# THE POTENTIAL BETWEEN PAIRS OF QUASI-SPHERICAL MOLECULES

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*Summary*

An attempt has been made to calculate the spherically smoothed intermolecular potential energy between the pairs $UF_6$–$UF_6$ and $SF_6$–$SF_6$ near their minimum potential separations. Very little difference was found in the radial forms of the potentials, apart from the scale factors of energy and length.

## I. INTRODUCTION

It has been suggested (Hamann and Lambert 1954*a*, 1954*b*, 1954*c*; Hamann 1955) that the potential between nearly spherical polyatomic molecules could be well represented by a spherically smoothed mutual potential energy of a (28,7) Lennard-Jones (1924) bireciprocal type. However, Rowlinson (1954) suggested that in the case of such pairs as $SF_6$–$SF_6$ and $UF_6$–$UF_6$ orientation effects are very important, and that the spherically smoothed potential would predict that the pair $UF_6$–$UF_6$ would be closer in behaviour to the inert gases, whereas, in fact, the reverse is true.

Consequently, it was decided to attempt the calculation of spherically smoothed (i.e. averaged over all orientations) mutual potential energies for these two pairs as a guide to their expected behaviour.

## II. CALCULATION

Taking the interaction potential energy between an atom $P$ of one molecule and an atom $Q$ of the other molecule as (Lennard-Jones 1938)

$$u_{PQ}=\varepsilon^*_{PQ}\left[\left(\frac{r^*_{PQ}}{r_{PQ}}\right)^{12}-2\left(\frac{r^*_{PQ}}{r_{PQ}}\right)^{6}\right], \quad \ldots\ldots\ldots\ldots \quad (1)$$

where $r_{PQ}$ is the distance between the nuclei of $P$ and $Q$ and $-\varepsilon^*_{PQ}$ is the minimum mutual potential energy corresponding to the separation $r_{PQ}=r^*_{PQ}$, the spherically smoothed interaction potential energy $U$ between two different quasi-spherical molecules $A_aB_b$ and $C_cD_d$, where $a,c=0$ or 1; $b,d>2$, can be shown to be

$$U=u_{AC}+v_{AD}+v_{BC}+w_{BD}, \quad \ldots\ldots\ldots\ldots\ldots \quad (2)$$

where $u_{AC}$ is the potential between the central atom of each molecule,
$v_{AD}$ is the potential between the atom $A$ and the shell of atoms $D$,
$v_{BC}$ is the potential between the atom $C$ and the shell of atoms $B$, and
$w_{BD}$ is the potential between the two shells of atoms $B$ and $C$.

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The expressions for $u_{AC}$, $v_{AD}$, and $v_{BC}$ were given by Hamann and Lambert (1954*a*). Their result for $w_{BD}$ should be replaced by

$$w_{BD}=\tfrac{1}{4}bd\varepsilon^*_{BD}\left\{\frac{r^{*12}_{BD}}{90\rho_{CD}\rho_{AB}r^{10}_{AC}}[(1+\alpha)^{-9}+(1-\alpha)^{-9}-(1+\beta)^{-9}-(1-\beta)^{-9}]\right.$$

$$\left.-\frac{r^{*6}_{BD}}{6\rho_{CD}\rho_{AB}r^{4}_{AC}}[(1+\alpha)^{-3}+(1-\alpha)^{-3}-(1+\beta)^{-3}-(1-\beta)^{-3}]\right\}, \quad .. \quad (3)$$

where

$$\alpha=\frac{\rho_{AB}+\rho_{CD}}{r_{AC}},\ \beta=\frac{\rho_{AB}-\rho_{CD}}{r_{AC}},$$

$r_{AC}$ is the distance between the centres of the molecules, and $\rho_{AB}$, $\rho_{CD}$ are the *A–B* and *C–D* bond lengths.

The above correction requires also that the expression for $w_{BB}/\varepsilon^*$ given in equation (**12**) of Hamann and Lambert's paper should be replaced by

$$\frac{w_{BB}}{\varepsilon^*}=\frac{8}{3}\left\{\frac{\zeta^{-10}}{15}[(1+\zeta^{-1})^{-9}+(1-\zeta^{-1})^{-9}-2]-\zeta^{-4}[(1+\zeta^{-1})^{-3}+(1-\zeta^{-1})^{-3}-2]\right\}. \quad \ldots\ldots\ldots\ldots\ldots \quad (4)$$

These corrections do not affect the remainder of Hamann and Lambert's paper.

For the potential $U(AB_6)$ between the pair $AB_6$–$AB_6$, these expressions reduce to

$$U(AB_6)=u_{AA}+2v_{AB}+w_{BB}, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

where

$$u_{AA}=\varepsilon^*_{AA}\left[\left(\frac{r^*_{AA}}{r_{AA}}\right)^{12}-2\left(\frac{r^*_{AA}}{r_{AA}}\right)^{6}\right],$$

$$v_{A_B}=3\varepsilon^*_{AB}\left\{\frac{r^{*12}_{AB}}{10r^{11}_{AA}\rho_{AB}}\left[\left(1-\frac{\rho_{AB}}{r_{AA}}\right)^{-10}-\left(1+\frac{\rho_{AB}}{r_{AA}}\right)^{-10}\right]\right.$$

$$\left.-\frac{r^{*6}_{AB}}{2r^{5}_{AA}\rho_{AB}}\left[\left(1-\frac{\rho_{AB}}{r_{AA}}\right)^{-4}-\left(1+\frac{\rho_{AB}}{r_{AA}}\right)^{-4}\right]\right\},$$

$$w_{BB}=9\varepsilon^*_{BB}\left\{\frac{r^{*12}_{BB}}{90r^{10}_{AA}\rho^2_{AB}}\left[\left(1+2\frac{\rho_{AB}}{r_{AA}}\right)^{-9}+\left(1-2\frac{\rho_{AB}}{r_{AA}}\right)^{-9}-2\right]\right.$$

$$\left.-\frac{r^{*6}_{BB}}{6r^{4}_{AA}\rho^2_{AB}}\left[\left(1+2\frac{\rho_{AB}}{r_{AA}}\right)^{-3}+\left(1-2\frac{\rho_{AB}}{r_{AA}}\right)^{-3}-2\right]\right\}.$$

$\rho_{SF}$, $\rho_{UF}$ are known from electron diffraction measurements, but the $\varepsilon^*$'s and $r^*$'s must be estimated. It was decided to use for $\varepsilon^*_{FF}$, $r^*_{FF}$, $\varepsilon^*_{SS}$, $r^*_{SS}$, $\varepsilon^*_{UU}$, $r^*_{UU}$ the figures for the inert gas next in atomic number, that is, Ne, A, Rn, respectively. For A, the values of $\varepsilon^*$ and $r^*$ are known; for Ne, $\varepsilon^*$ and $r^*$ were estimated from critical constants; and for Rn, $\varepsilon^*$ was estimated from critical constants, and for $r^*$ a calculated figure for crystal distances was used (see Table 1).

TABLE 1

CRITICAL CONSTANTS AND MOLECULAR PARAMETERS

Critical constants are from *International Critical Tables* **3**: 248 ff

| Gas | Critical Temperature, $T_c$ (°K) | Critical Pressure, $p_c$ (dynes cm$^{-2}\times10^{6}$) | Critical Volume, $V_c$ (cm$^3$ mole$^{-1}$) | $\varepsilon^*/k$ (°K) | $r^*$ (cm$\times10^{-8}$) |
|---|---|---|---|---|---|
| Ne | 44·46 | 26·243 | 41·70 | 34·8†‡ | 3·19†‡ |
| A | 151·16 | 48·636 | 75·22 | 119·7§ | 3·83§ |
| Rn | 377·7 | — | — | 295·1‡ | 4·48‖ |

† The above figures for Ne were those used in the calculations. Dr. S. D. Hamann has pointed out that Corner (1948) gives the figures $\varepsilon^*/k$, 36·3 °K; $r^*$, $3\cdot16\times10^{-8}$ cm.

‡ Calculated from critical constants.

§ Bird, Spotz, and Hirschfelder (1950).

‖ Calculated interatomic distance in crystal, Sarkisov (1948).

The calculations from the critical constants were based on the mean figures (Hamann and Lambert 1954*b*, p. 23, Table 3)

$$V_c=3\cdot02V_0,\quad T_c=1\cdot28T_0,\quad p_c=0\cdot125p_0,$$

where $V_c$, $T_c$, $p_c$ are the critical volume, temperature, and pressure respectively, and $V_0$, $T_0$, $p_0$ are the "molecular" units of volume, temperature, and pressure given by

$$\left.\begin{aligned} V_0&=2^{-\frac{1}{2}}Nr^{*3},\\ T_0&=\varepsilon^*/k,\\ p_0&=2^{\frac{1}{2}}\varepsilon^*/r^{*3},\end{aligned}\right\}\qquad\ldots\ldots\ldots\ldots\ldots\quad(6)$$

where $N$ is Avagadro's number and $k$ is Boltzmann's constant.

For $\varepsilon^*_{SF}$, $r^*_{SF}$, $\varepsilon^*_{UF}$, $r^*_{UF}$ the averaging rules suggested by Guggenheim and McGlashan (1951) were used:

$$\varepsilon^*_{AB}=(\varepsilon^*_{AA}\varepsilon^*_{BB})^{\frac{1}{2}},\qquad r^*_{AB}=\tfrac{1}{2}(r^*_{AA}+r^*_{BB})$$

(see Table 2).

TABLE 2

MOLECULAR PARAMETERS

| Pair of Atoms | $\varepsilon^*/k$ (°K) | $r^*$ (cm$\times10^{-8}$) | $\rho$ (cm$\times10^{-8}$) |
|---|---|---|---|
| F–F | 34·8 | 3·19 | — |
| S–S | 119·7 | 3·83 | — |
| U–U | 295·1 | 4·48 | — |
| S–F | 64·5 | 3·51 | 1·57 |
| U–F | 101·3 | 3·84 | 1·78 |

If the distance between the centres of the molecules $AB_6$–$AB_6$ be denoted by $R(AB_6)$, which, of course, is equal to $r_{AA}$, then the minimum mutual potential

energy $-E^*(AB_6)$ occurs at the separation $R(AB_6)=R^*(AB_6)$. The calculated values of $R^*$ and $E^*$ are given in Table 3.

TABLE 3

MOLECULAR PARAMETERS AND CRITICAL CONSTANTS

| Gas | $R^*$ (cm $\times 10^{-8}$) | $E^*/k$ (°K) | $T_c$ (°K) | $p_c$ (dynes cm$^{-2}$ $\times 10^6$) | $T_c/T_0$ | $p_c/p_0$ |
|---|---|---|---|---|---|---|
| $SF_6$ | 5·44† | 311† | 319‡ | 37·5‡ | 1·03 | 0·099 |
| $UF_6$ | 5·74† | 485† | 503§ | 46·1§ | 1·04 | 0·092 |

† Calculated value.

‡ I.C.I. Gen. Chem. Div. Rep. No. G.C.S. 15258.

§ Oliver, Milton, and Grisard (1953).

The potential was calculated in each case over the interval

$$0\cdot92<R(AB_6)/R^*(AB_6)<1\cdot12$$

and the curves for $U/E^*(AB_6)$ against $R(AB_6)/R^*(AB_6)$ plotted (see Fig. 1). For comparison, the (12,6) and (28,7) bireciprocal potentials are plotted on the same figure.

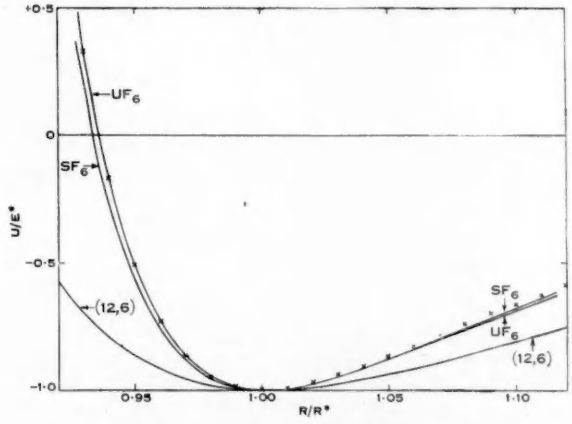


Fig. 1.—Intermolecular mutual potential energies: Curves are given for the pairs $SF_6$–$SF_6$ and $UF_6$–$UF_6$ as well as for the Lennard-Jones (12,6) potential. The crosses represent points on the curve for the (28,7) potential.

## III. CONCLUSION

It will be seen that there is very little difference between the two curves for $U/E^*$ against $R/R^*$, and that both are quite well approximated to by the (28,7) curve.

In other words, the spherically smoothed potential energy calculations would not lead one to expect much difference in the " reduced " properties of the pairs $UF_6$–$UF_6$ and $SF_6$–$SF_6$, that is, their properties should be similar when expressed in terms of the molecular units referred to above. The reduced critical constants in Table 3 support this expectation.

## IV. Acknowledgments

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# THE OXIDATION OF CARBON WITH ATOMIC OXYGEN

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*Summary*

Atomic oxygen, produced by dissociation of molecular oxygen in a radio frequency field, will react with amorphous or graphitic carbon at room temperatures and both carbon monoxide and carbon dioxide appear in the product gases. Carbon monoxide appears to be the primary product of oxidation of carbon, the carbon dioxide being produced by direct combination of carbon monoxide with oxygen which takes place mainly at the carbon surface. Atomic oxygen will also react with carbon dioxide to produce carbon monoxide and molecular oxygen but the quantity of carbon monoxide produced by this reaction is small compared to that produced by direct oxidation of the carbon.

## I. Introduction

When oxygen reacts with carbon the gaseous products are carbon monoxide and carbon dioxide. There still exists considerable confusion in the understanding of the oxidation mechanism and it is not clear whether only one, or both, of the gases are produced as primary products. It has been suggested by many workers (Strickland-Constable 1944; Arthur, Bangham, and Thring 1949; Mertens 1950, 1954) that the primary reaction is the formation of carbon monoxide. The exact mechanism is not clear but it appears that the oxygen is chemisorbed at the carbon surface and is liberated as carbon monoxide.

If the attached oxygen is present in the atomic form (Gauger and Zelinski 1951) carbon monoxide could be formed readily and the suggestion by these authors that oxygen may be produced in the atomic state at the carbon surface would lead to the idea that carbon monoxide may be the primary and perhaps the only product of direct oxidation.

Experiments have shown (Mayer 1938) that, as the partial pressure of oxygen is decreased, so is the production of carbon monoxide favoured. The work of van Loon and Smeets (1950) and Karzhavina (1938) indicates a similar trend in that an increase in the streaming rate of oxygen over a heated carbon surface tended to produce more carbon monoxide.

In contrast, other workers (Lowry and Hulett 1920; Meyer 1932; Chukhanov and Karzhavina 1939, 1940) have suggested that both carbon monoxide and carbon dioxide are primary products. Their suggestions involve the formation of complex oxygen compounds on the carbon surface with subsequent breakdown to the two oxides of carbon. The kinetic interpretation of

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these results would indicate that more than one reaction is contributing to the overall result and that oxidation, as observed, is complicated by secondary reactions.

It has been known for some years that oxygen (as well as other gases, e.g. $H_2$, $Cl_2$, etc.) can be obtained in the atomic form at pressures below about 10 mm Hg by the action of an electric discharge. Such discharges have usually been obtained by connecting a high voltage A.C. source across two electrodes sealed into the system. A number of reactions of atomic gases has been investigated (see, for example, Geib 1936) including some involving atomic oxygen. Qualitative experiments were made by Sihvonen (1932, 1933*a*, 1933*b*) using graphite and oxygen at pressures of $3–15 \times 10^{-3}$ mm Hg. The gas or the graphite itself was subjected to X-radiation, or a discharge was passed between a platinum electrode and a graphite electrode. However, no real distinction could be made between gases previously chemisorbed on the carbon material and those possibly formed by reaction with the oxygen. Both carbon monoxide and carbon dioxide were observed in the product gases.

The following work was done in an effort to clarify the state of knowledge on the oxidation of carbon with atomic oxygen. It stemmed from the observation that graphite is rapidly oxidized by atomic oxygen at, or even below, ambient room temperatures and at pressures up to 10 mm Hg.

## II. Experimental

### (*a*) *Raw Materials*

The carbon used was a sample of purified natural graphite containing 0·02 per cent. ash. A sample of wood char prepared from *Eucalyptus marginata* by heating in nitrogen at 750 °C and industrial diamond were also used in some experiments. The gases were obtained from cylinders and were of commercial quality free from impurities except nitrogen which varied from traces to as high as 1 per cent.

### (*b*) *Gas Analyses*

These were done in a gas chromatograph using a silica gel column at atmospheric temperature with hydrogen as the carrier gas.

### (*c*) *Apparatus*

An alternative method for producing atomic oxygen etc. is to apply to the gas a high frequency electromagnetic field. This has the advantage that no electrodes are needed and hence no foreign materials are introduced into the reaction system. Furthermore, the waveform of the oscillations may be sinusoidal, the power may be varied continuously over a wide range, and the frequency, which may easily be maintained constant, can be chosen and readily changed from a few kilocycles per second to several hundred megacycles per second.

The radio frequency source used was a type 250TH vacuum tube operated as a power amplifier, and excited by a crystal controlled generator. For the work described in the present paper, a frequency of 30 mc/s was employed. The power was controlled by means of a Variac in the primary of the high voltage

supply to the power amplifier and the tuning coil in the plate circuit of the power amplifier was wound around the tube carrying the oxygen, as shown in Figure 1.

Figure 1 (*a*) shows the apparatus as arranged for general use, and Figure 1 (*b*) shows the modifications made to it for the study of the reaction between graphite and atomic oxygen. Vacuum needle valves controlled the flow of the gases, the lengths of capillary tubing serving to prevent the atomic gases diffusing back to the valves. The apparatus was constructed of Pyrex glass using Quickfit joints lubricated with silicone vacuum grease.

The vacuum pump was modified to minimize the dead space on the outlet side and to allow samples of gas to be collected over mercury.

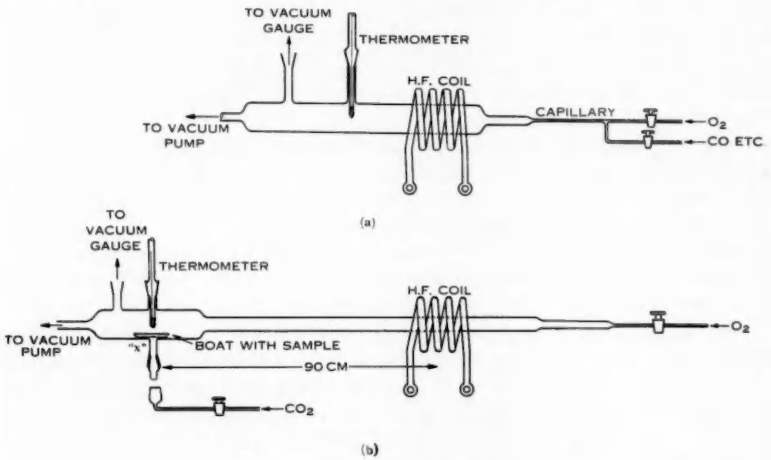


Fig. 1.—General scheme of apparatus.

The concentration of oxygen atoms was measured by the calorimetric method described by Poole (1937). A polished copper coil was inserted into the tube, and the inlet and outlet temperatures of water flowing through the coil were measured by means of differential copper-constantan thermocouples.

### (*d*) *Reactions Studied*

In investigating the reaction of carbon and atomic oxygen, four reactions were considered :

(i) The reaction of carbon monoxide and atomic oxygen.
(ii) The possible breakdown of carbon dioxide due to the field.
(iii) The main reaction between carbon and atomic oxygen.
(iv) The possible breakdown of carbon dioxide due to atomic oxygen.

(i) The reaction between carbon monoxide and oxygen atoms has been stated to proceed only at a very slow rate (Harteck and Kopsch 1931). In the present study a fixed pressure of 0·1 mm Hg was used and a mixture of equal

volumes of carbon monoxide and oxygen was passed through the system. Under these conditions, in the all-glass apparatus, 5 per cent. carbon dioxide was present in the effluent gas but when a small amount of platinum black catalyst was present about 20 cm downstream from the coil, this increased to 12 per cent. carbon dioxide.

These results indicated that the homogeneous reaction could not contribute a significant amount of carbon dioxide at pressures below 0·1 mm Hg, particularly as the amount would progressively diminish with reducing pressure. It was also apparent that an active surface could promote the formation of carbon dioxide, and it is believed (see below) that carbon itself probably acts very efficiently in this regard.

(ii) When pure carbon dioxide was passed through the apparatus it was found that the following reactions could occur:

$$CO_2 \rightarrow CO + (O), \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

$$(O) + (O) \rightarrow O_2. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

The extent to which carbon dioxide broke down to carbon monoxide and oxygen depended on the pressure in the manner shown in curve 1, Figure 2. It is seen that with the field strength used the breakdown was very small even at pressures as low as 0·1 mm Hg. Thereafter it increased fairly rapidly until at 0·03 mm Hg 75 per cent. of the carbon dioxide was converted to carbon monoxide.

This reaction was accompanied by the bright light-blue luminescence characteristic of excited carbon monoxide molecules.

(iii) The main reaction, between carbon and atomic oxygen: If the breakdown of carbon dioxide as described above, occurred during the study of the carbon–atomic oxygen reaction, erroneous results would be obtained and consequently steps were taken to prevent any of the carbon dioxide formed from being subjected to the influence of the field. This was achieved by lengthening the working tube so that, over the whole range of pressures used, gases arising from the carbon were unable to diffuse far enough back against the oxygen stream to reach the coil. The distance between the carbon sample and coil, necessary to ensure this, was 90 cm. The concentration of oxygen atoms available at this distance, for various pressures and an input of 130 W to the amplifier, is shown in Table 1.

TABLE 1

CONCENTRATION OF OXYGEN ATOMS AT CARBON SAMPLE

| | | | |
|---|---|---|---|
| Pressure (mm Hg) .. .. .. | 0·15 | 0·07 | 0·05 |
| Volume $O_2$ (c.c./hr at N.T.P.) .. | 440 | 140 | 100 |
| $O_2$ in atomic form (%) .. .. | 18 | 34 | 90 |
| $O_2$ reacted with C (%) .. .. | 13 | 35 | 70 |

Graphite samples (4–5 g) were placed in the boat with the apparatus arranged as in Figure 1 (*b*). Oxygen was passed at total internal pressures of from 0·03 to 0·2 mm Hg and the percentage of the oxygen that reacted with the carbon

is shown in Table 1. The results of these experiments are shown in curve 2, Figure 2, in which $CO/CO_2$ ratios are plotted against both volume of oxygen passing and the pressure. At a pressure of 0·16 mm Hg the $CO/CO_2$ ratio was 0·46 : 1, but as the pressure was decreased carbon monoxide became predominant and at 0·04 mm Hg the ratio was 20 : 1. During these experiments a blue luminescence resembling that of excited carbon monoxide was observed in the vicinity of the graphite at higher pressures, and extending back towards the coil at lower pressures. This pointed to excitation of carbon monoxide, due

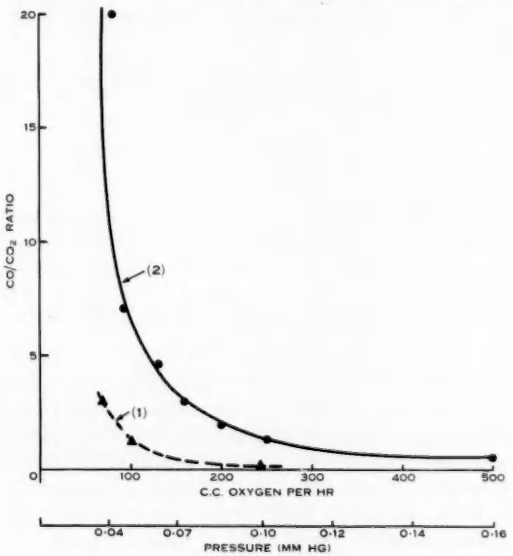


Fig. 2.—Curve 1 showing the effect of high frequency field on the $CO/CO_2$ ratio for carbon dioxide passing through the field at different pressures. Curve 2 the effect of pressure on the $CO/CO_2$ ratio for the products of reaction of carbon and atomic oxygen.

probably to oxygen atoms. As the pressure was lowered the intensity of the luminescence increased. This is in contrast to the luminescence occurring during the reaction

$$CO+(O)+M \rightarrow CO_2^*+M, \quad \ldots\ldots\ldots\ldots \quad (3)$$

which is stated by Broida and Gaydon (1953) to weaken rapidly at low pressures. The luminescence raised the question as to whether oxygen atoms could cause breakdown of carbon dioxide in a manner similar to that obtained in the field. It was decided to determine if this was the case.

It is of interest to note here that atomic oxygen reacted with amorphous carbon more readily than with graphite, but showed very little reactivity with diamond even at a temperature of 200 °C. It should also be noted that the

percentage of the total oxygen reacting with the carbon is in good agreement with the percentage found to be in the atomic form. This suggests that only atomic oxygen takes part in the oxidation.

(iv) The reaction $CO_2+(O)$: When excess oxygen atoms are present this reaction may be regarded in one sense as the reverse of reaction (**1**), that is,

$$CO+(O)\rightarrow CO_2, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

and

$$CO_2+(O)\rightarrow CO+O_2. \quad \ldots\ldots\ldots\ldots\ldots \quad (5)$$

It is likely that under given conditions of pressure etc. a steady state may be reached.

Carbon dioxide was introduced into the system at the point " x " in Figure 1(*b*), that is, at the position normally occupied by the carbon sample, and an excess of oxygen was passed through the field. Experiments were done at total pressure between 0·04–0·15 mm Hg. A small fraction of the carbon dioxide was decomposed, but in no case was the $CO/CO_2$ ratio greater than 0·3 : 1. These experiments showed that this reaction could not account for the high $CO/CO_2$ ratios obtained in Section II (*d*) (iii).

## III. Discussion

The preceding results show that the oxidation of carbon by atomic oxygen results in the formation of both carbon monoxide and carbon dioxide. With decreasing pressure, the amount of carbon monoxide increases very rapidly, and curve 2, Figure 2, indicates that, as the pressure is decreased to a limiting value, the product gas would consist essentially of carbon monoxide. At higher pressures the presence of carbon dioxide may indicate either a secondary reaction in which carbon monoxide is converted to carbon dioxide, or the formation of carbon dioxide as a primary product.

It is clear from the experimental results that carbon dioxide can be broken down in a radio frequency field to produce carbon monoxide. However, as shown in curve 1, Figure 2, when carbon dioxide was passed through the field the $CO/CO_2$ ratio was much lower for given conditions of pressure and field than in the oxidation of carbon, where precautions were taken to prevent products of oxidation returning to the field. This suggests that if carbon dioxide was produced as a primary product, some of it would be converted to carbon monoxide if subjected to the field. However, since the quantity of carbon monoxide present is much greater than could be produced by the splitting of carbon dioxide, it must be assumed that carbon monoxide is a primary product. When the carbon dioxide is far enough from the field, as demonstrated by introduction of this gas into the atomic oxygen stream at the position previously occupied by the sample, the amount of carbon dioxide converted is only small and in the pressure range considered the $CO/CO_2$ ratio did not exceed 0·3 : 1.

Carbon dioxide can also be formed by the secondary reaction between carbon monoxide and atomic oxygen. The amount of carbon dioxide formed in this way is only small and normally would not account for the proportion found in the product gases during oxidation at higher pressures. However, when a

catalytic surface such as platinum black was present, the amount of carbon dioxide was greatly increased. It has been suggested (Broida and Gaydon 1953) that the oxidation of carbon monoxide by atomic oxygen may be catalysed by the presence of a third body surface. This could explain the larger proportions of carbon dioxide appearing in the oxidation products at higher pressures. As the pressure is decreased, the mean free path becomes greater and the number of carbon monoxide molecules returning to the carbon surface to be converted to carbon dioxide becomes less. The relationship between mean free path and pressure is of a hyperbolic form, as is the $CO/CO_2$ ratio *v.* pressure, shown in Figure 2, curve 2, and it might be inferred that this could account for the greater proportion of carbon monoxide appearing at lower pressures. This is in agreement with experiments of Meyer (1932), van Loon and Smeets (1950), and Chukhanov (1938*a*, 1938*b*), who showed that, with increasing gas velocity through a carbon bed, when the chance of product gases reacting at the carbon surface is decreased, the product gases were predominantly carbon monoxide and extrapolation to high velocities showed that the ratio $CO/(CO+CO_2)$ approached unity.

The fact that atomic oxygen will react readily with carbon at ambient room temperatures may indicate that the normal process of oxidation is connected with the production of atomic oxygen. This suggestion is supported by the fact that at any pressure the percentage of oxygen combined with the carbon corresponds to the proportion of oxygen available in the atomic form, as shown in Table 1. Carbon, particularly high temperature carbons such as graphite, react only slowly with oxygen at temperatures below 300–500 °C but as the temperature is increased, and consequently the concentration of atomic oxygen, reaction becomes faster and eventually self-supporting due to the exothermal nature of the reaction.

Experiments with different carbons indicated that oxygen attack may begin at certain specific sites. The exact nature of these sites is not known. However, with relatively impure carbon, obtained by charring wood, the rate of oxidation was much greater than with graphite of high purity, whereas when diamond was used the reaction was extremely slow. It may be that attack begins at the edge of the carbon lattice where free electrons are available, as suggested by Long and Sykes (1948) and Strickland-Constable (1950), and that oxygen with its two unpaired electrons would be readily attached at these positions. The active sites may also be associated with specific groups, such as oxygen compounds in the carbon, as suggested by Blackwood (1959). Either of these explanations could account for the decreasing reactivity as we pass from amorphous carbon through graphite to diamond.

## IV. Acknowledgments

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# SOME STUDIES IN INORGANIC COMPLEXES

## IV. TITANIUM(III) AND TITANIUM(IV)

By G. J. SUTTON*

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*Summary*

The titanium complexes $TiCl_3.D.H_2O$, $TiBr_3.D.H_2O$, $TiCl_4.D$, and $TiBr_4.D$, in which D is *o*-phenylenebisdimethylarsine, have been prepared and studied. By means of conductivity and molecular weight measurements, it has been shown that they are 6-covalent non-electrolytes. The thiocyanato complexes $[NH_4][Ti(SCN)_4(H_2O)_2]$, $[NH_4][Ti(SCN)_4]$, and $[NH_4][Ti(SCN)_4OH.H_2O]$ were also prepared and their structures verified by conductivity measurements.

### I. INTRODUCTION

With a lone electron in a 3*d* orbital titanium(III) is readily oxidized to the quadrivalent state and very few stable complexes of titanium(III) are known. Of the compounds investigated, the fluoro complex $K_3TiF_6$ has almost a normal magnetic moment of 1·70 Bohr magnetons (B.M.) at low temperatures, according to Raychaudhuri and Sengupta (1936). The simple chloride at 20 °C has been reported as having a moment of 1·78 B.M. by Wedekind and Hausknecht (1913), 1·42 B.M. by Starr, Bitter, and Kaufmann (1940), and 1·61 B.M. by Klemm and Krose (1947). The latter workers obtained values of about 1·25 and 0·94 B.M. respectively for the corresponding bromide and iodide, and having made a comprehensive study of the three halides over a wide range of temperature they showed that the compounds are antiferromagnetic at temperatures below 60 °C. More recently, Adler and Selwood (1954) reported that the oxide $Ti_2O_3$ is also antiferromagnetic.

The deep violet colour formed by allowing thiocyanate and aqueous titanium(III) to react was observed by van der Pfordten (1886) and the formula $M_3Ti(SCN)_6.6H_2O$ was assigned to the potassium and ammonium salts. Since the anion was described as being a stable complex, it was decided to investigate the magnetic moment of the ammonium salt. Furthermore, although titanium(III) is not readily coordinated by less negative atoms, it was decided to attempt the formation of complexes with the chelating ligand *o*-phenylenebisdimethylarsine ; for besides having vacant 3*d* orbitals for $\sigma$ bond formation the titanium atom may feed 3*p* electrons into the vacant 4*d* orbitals of the arsenic atom. It was considered that using this ligand the possibility of metal-metal bonding by the single 3*d* electrons would be offset by magnetic dilution.

Repetition of the work of van der Pfordten failed to yield a pure sample of the violet thiocyanate complex from aqueous solution. The extraction of the violet complex by diethyl ether was described by van Pascheke and Schaller

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(1938), and Bock (1951) showed that the maximum partition coefficient for the complex occurs at a concentration of 0·1 mole of titanous chloride and about 5·0 moles of ammonium thiocyanate per litre respectively. Accordingly, the complex was extracted with peroxide-free ether, and by the addition of light petroleum and evaporation *in vacuo* violet crystals of a salt of composition corresponding to the formula $[NH_4][Ti(SCN)_4(H_2O)_2]$ were obtained. The structure was confirmed by conductivity measurements in nitrobenzene which showed that the substance is a uni-univalent electrolyte. Evidence for the presence of aquo molecules in the 6-covalent anion complex was supported by shaking the ethereal solution with solid calcium chloride, when an orange solution resulted, from which the anhydrous salt $[NH_4][Ti(SCN)_4]$ was obtained. The latter substance was shown to be a uni-univalent electrolyte by conductance measurements. Anhydrous sodium sulphate or magnesium sulphate were also effective in discharging the purple colour of the original complex. Both complexes were found to give magnetic moments at 20°C of about 1·0 B.M. and here again antiferromagnetism is indicated. By shaking the orange anhydrous complex in ether with water or aqueous ethanol the deep purple colour was regenerated. It is likely that due to lability the aquo molecules are interchangeable with ether molecules, although no complex containing the latter was isolated. It is interesting to note that brownish red oxalato complexes of the type $MTi(C_2O_4)_2.2H_2O$, in which M is K, Rb, or $NH_4$, were described by Schumb and Sundström (1938). Attempts to precipitate the violet complex from aqueous solution using triphenylmethylarsonium and tetramethylammonium cations were unsuccessful, simple thiocyanates being precipitated in both cases. The orange complex ammonium tetrathiocyanatotitanate(III) was also prepared by dissolving hexa-aquotitanium(III) chloride in acetone, and after refluxing for a few minutes treating with ammonium thiocyanate in acetone solution. It is noteworthy that van der Pfordten observed the replacement of aquo molecules by halogen or pseudohalogen with titanium(III) in non-aqueous solutions. Both the aforementioned complexes are oxidized by dissolved oxygen or by peroxides to a yellow titanium(IV) complex $[NH_4][Ti(CNS)_4OH.H_2O]$. This substance is diamagnetic and conductivity measurements in nitrobenzene confirmed its structure as a uni-univalent electrolyte. Ionic weight determinations of these complexes were not carried out for reasons of low solubility in suitable solvents. During these investigations no evidence of the black titanium(III)thiocyanate substance reported by Grossman (1906) was found. Attempts to form thiocyanate complexes of titanium(II) by electrolytic reduction were unsuccessful, and this was not unexpected since the reduction of thiocyanate to cyanide and hydrogen sulphide by titanium(II) was observed by van der Pfordten (1886) and later by van Pascheke and Schaller (1938).

Due to its high polarizing power titanium(III) does not readily form complexes with less negative elements such as nitrogen as the donor atom. However, it was found that coordination takes place in glacial acetic acid with the chelating ligand *o*-phenylenebisdimethylarsine with the formation of red precipitates of the type $TiX_3.D.H_2O$, in which X is chlorine or bromine and D is the diarsine. It is interesting to note that in this case water occupies the

6th coordinating position and that no product is obtained when acetic anhydride is present. The water molecule is derived from the hexa-aquotitanium(III) salt used in the preparation. The red chloro or bromo complexes are rapidly decomposed by water or by solvents capable of forming hydroxyl groups, but they are soluble in benzene forming deep orange solutions, from which they may

TABLE 1

MAGNETIC SUSCEPTIBILITIES CORRECTED TO 20 °C

| Substance | $\chi_g \times 10^{-6}$ | $\chi_M \times 10^{-6}$ | $\chi_{M'} \times 10^{-6}$ | $\mu$ (B.M.) |
|---|---|---|---|---|
| $[NH_4][Ti(SCN)_4(H_2O)_2]$ | 0·770 | 257 | 425 | 0·99 |
| $[NH_4][Ti(SCN)_4]$ .. | 0·905 | 270 | 412 | 0·99 |
| $[NH_4][Ti(SCN)_4OH.H_2O]$ | —0·216 | —72 | 90 | 0 |
| $TiCl_3.D.H_2O$ .. .. | 0·173 | 79 | 347 | 0·91 |
| $TiBr_3.D.H_2O$ .. .. | 0·218 | 129 | 330 | 0·88 |
| $TiCl_4.D$ .. .. .. | —0·453 | —216 | 59 | 0 |
| $TiBr_4.D$ .. .. .. | —0·398 | —260 | 51 | 0 |

be precipitated by the addition of light petroleum. Cryoscopic molecular weight determinations in benzene showed that the compounds are monomeric and conductivity determinations in nitrobenzene confirmed their structure as 6-covalent non-electrolytes. The magnetic moments were found to be about 0·9 B.M. at 20 °C and it is likely that this is again due to antiferromagnetism.

TABLE 2

MOLECULAR CONDUCTIVITIES AT 25 °C AND MOLECULAR WEIGHT DETERMINATIONS

| Substance | *M* Conductivities | | Molecular Weights | | |
|---|---|---|---|---|---|
| | (mhos) | *M* Conc. | Found | % Conc. | Calc. |
| $[NH_4][Ti(SCN)_4(H_2O)_2]$ | 18·3 | 0·010 | | | |
| $[NH_4][Ti(SCN)_4]$ .. | 18·9 | 0·003 | | | |
| $[NH_4][Ti(SCN)_4OH.H_2O]$ | 21·4 | 0·003 | | | |
| $TiCl_3.D.H_2O$ .. .. | 0·53 | 0·006 | 407 | 1·01 | 458 |
| $TiBr_3.D.H_2O$ .... .. | 0·31 | 0·011 | 514 | 1·32 | 592 |
| $TiCl_4.D$ .. .. .. | 0·90 | 0·007 | 464 | 1·93 | 476 |
| $TiBr_4.D$ .. .. .. | 1·3 | 0·006 | 602 | 1·76 | 654 |

The complexes are readily oxidizable to the quadrivalent state by halogen in benzene solution, forming non-electrolytes of the type $TiX_4.D$. The latter complexes are precipitated from benzene solution as golden plates, which are diamagnetic and have a negligible conductivity in nitrobenzene. Molecular weight determinations in benzene by the cryoscopic method confirmed the structure of these complexes as 6-covalent monomers.

The magnetic susceptibilities of the foregoing complexes are summarized in Table 1, whilst molecular conductivities in A.R. nitrobenzene at 25 °C and molecular weight determinations by the cryoscopic method are given in Table 2.

## II. Experimental

The molecular conductivity measurements and nitrobenzene purification were carried out according to the procedures outlined previously. The diethyl ether was freed from peroxide by shaking with ferrous sulphate solution and redistilling. Benzene and light petroleum were treated with anhydrous sodium sulphate, then sodium wire, and redistilled.

(*a*) *Ammonium Tetrathiocyanatodiaquotitanate(III).*—Hexa-aquotitanium(III) chloride (6·55 g) in water (53 ml) was treated with ammonium thiocyanate (11·4 g) in water (50 ml) and the deep violet product extracted with ether (80 ml) in 2 portions. The combined ethereal solutions were filtered, evaporated to one-third volume on a water-bath in an atmosphere of carbon dioxide, and light petroleum (2 ml) added with shaking. Further evaporation was carried out in a vacuum desiccator until a reddish violet powder resulted. This was separated by decantation and dried in a vacuum desiccator over concentrated sulphuric acid (yield 4·5 g) (Found: $NH_3$, 5·1; CNS, 70·0; Ti, 13·9%. Calc. for $NH_4Ti(SCN)_4.2H_2O$: $NH_3$, 5·1; CNS, 69·5; Ti, 14·3%).

(*b*) *Ammonium Tetrathiocyanatotitanate(III).*—The aforementioned procedure was repeated and the ethereal extract shaken with calcium chloride (10 g) until the violet colour had changed to an orange colour. The solution was filtered and after the addition of light petroleum (2 ml) allowed to stand in a corked flask with a minimum air space in a refrigerator. After 24 hr the orange powdery precipitate which formed was separated by decantation and filtration and dried in a vacuum desiccator (yield 6·8 g) (Found: $NH_3$, 5·7; CNS, 78·5; Ti, 15·8%. Calc. for $NH_4Ti(SCN)_4$: $NH_3$, 5·7; CNS, 77·9; Ti, 16·1%).

(*c*) *Ammonium Tetrathiocyanatoaquohydroxytitanate(IV).*—A further preparation of the latter substance (*b*) (5 g) was dissolved in water (60 ml) and sufficient 2% hydrogen peroxide solution added. The mixture was evaporated to 10 ml, the yellow powdery solid removed by filtration and dried in a vacuum desiccator over concentrated sulphuric acid (yield 5·4 g) (Found: $NH_3$, 4·9; CNS, 69·3; Ti, 14·6%. Calc. for $NH_4TiOH(SCN)_4.H_2O$: $NH_3$, 5·1; CNS, 69·7; Ti, 14·4%).

(*d*) *Trichloromono-(o-phenylenebisdimethylarsine)monoaquotitanium(III)*—An aqueous solution of hexa-aquotitanium(III) chloride (12½%) was evaporated to dryness in an atmosphere of carbon dioxide and the violet solid crushed and transferred to a vacuum desiccator over sulphuric acid. Portion of the salt (0·80 g) was dissolved in glacial acetic acid (40 ml) with warming and any insoluble matter including titanic acid filtered off. The diarsine (0·58 g) was added slowly to the solution with stirring and the solution warmed to about 80 °C. The red flocculent precipitate which formed was removed by centrifuging and dissolved in sufficient anhydrous benzene with warming. Light petroleum was then added to the deep orange solution with shaking and the red precipitate which formed separated by centrifuging. The complex was dried in a vacuum desiccator (yield 0·57 g). The substance was found to decompose at 205 °C (Found: C, 26·5; H, 4·0; Cl, 22·8%. Calc. for $C_{10}H_{18}As_2Cl_3OTi$: C, 26·2; H, 4·0; Cl, 23·2%).

(*e*) *Tribromomono-(o-phenylenebisdimethylarsine)monoaquotitanium(III).*—The latter experimental procedure was repeated, but lithium bromide (1·0 g) was added to the acetic acid solution before the addition of diarsine. The deep red complex was found to decompose at 206 °C (yield 0·63 g) (Found: C, 19·8; H, 2·6; Br, 41·0%. Calc. for $C_{10}H_{18}As_2Br_3OTi$: C, 20·2; H, 3·0; Br, 40·5%).

(*f*) *Tetrachloromono-(o-phenylenebisdimethylarsine)titanium(IV).*—A further preparation of the chloro complex $TiCl_3.D.H_2O$ (0·42 g) was dissolved in benzene (10 ml) and a slight excess of chlorine bubbled slowly through the solution. The latter was allowed to stand at about 10 °C for 24 hr, when a pale golden crystalline precipitate settled out. This was separated by centrifuging, washed once with light petroleum, and dried in a vacuum desiccator (yield 0·40 g) (Found: C, 25·4; H, 4·0; Cl, 30·1%. Calc. for $C_{10}H_{16}As_2Cl_4Ti$: C, 25·2; H, 3·4; Cl, 29·8%).

(*g*) *Tetrabromomono-(o-phenylenebisdimethylarsine)titanium(IV).*—The latter procedure was repeated using an additional preparation of the bromo complex $TiBr_3.D.H_2O$ (0·50 g) and adding bromine (0·1 g) to the solution in benzene (10 ml). The golden crystalline precipitate was dried *in vacuo* (yield 0·47 g) (Found: C, 18·4, 18·1; H, 3·1, 2·9; Br, 48·4%. Calc. for $C_{10}H_{16}As_2Br_4Ti$: C, 18·4; H, 2·5; Br, 48·9%).

In the foregoing, the halogen and thiocyanate were estimated with silver ion, the titanium gravimetrically as titanium dioxide, and the ammonia by Kjeldahl distillation.

## III. Acknowledgment

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# THE EFFECT OF COBALT ON THE KINETICS OF OXYGEN EVOLUTION AT A LEAD DIOXIDE ANODE IN SULPHURIC ACID

By D. F. A. Koch*

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*Summary*

The overpotential ($\eta$)-log current density (log $i$) curves for the evolution of oxygen at a lead dioxide anode in 2N $H_2SO_4$ both in the absence and presence of cobaltous sulphate in solution have been used to determine the electrode kinetic constants $\alpha$; $i_0$ for a series of temperatures and also $\Delta H_0^*$. At 25 °C in the absence of cobalt $\alpha = 0{\cdot}59$, $i_0 = 10^{-11}$, and $\Delta H_0^* = 15$ kcal mole$^{-1}$. When 13 mg/l cobaltous sulphate is added $\alpha = 1{\cdot}0$, $i_0 = 10^{-15}$, and $\Delta H_0^* = 29$ kcal mole$^{-1}$.

Possible mechanisms for the reaction are discussed on the basis of these values and the rate determining steps suggested (where M represents the $PbO_2$ surface) are $M + H_2O = MOH + H^+ + e$ in the absence of cobalt and $2CoOH^{++} = 2Co^{++} + H_2O + O$ in its presence.

## I. Introduction

The lead dioxide electrode is of fundamental importance in the lead accumulator and is also used extensively as an insoluble anode for the electrowinning of metals such as copper and zinc. In common with other inert anodic material lead dioxide has a large overpotential for oxygen evolution and consequently the cell voltage for electrowinning is considerably higher than required on thermodynamic grounds for the deposition of metal at the cathode and evolution of oxygen at the anode. It has been shown by Wark (1929) and Koenig, MacEwan, and Larsen (1941) that small concentrations of cobaltous sulphate in a solution of sulphuric acid and zinc sulphate used for zinc electrowinning considerably reduce this overpotential. The work described here is an extension of a potential-time study on the effect of cobaltous sulphate in 2N $H_2SO_4$ on the formation of a lead dioxide surface on a lead anode (Koch 1958) where it was shown that the lead dioxide film formed in the presence of cobalt is much thinner than in its absence and also that a reproducible anode surface was more readily prepared when cobalt was present in solution. In the following work a study of the overpotential ($\eta$)-current density ($i$) relationships have given some electrode kinetic parameters for oxygen evolution in the presence and absence of cobalt and the values of these parameters have been used to help decide the mechanism of the reaction.

A linear relationship between $\eta$ and log $i$ for activation overpotential was observed by Tafel (1905)

$$\eta = a + b \log i. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

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Application of absolute rate theory to an electrode reaction (Glasstone, Laidler, and Eyring 1941) shows that this expression may be written in the form

$$\eta = \frac{RT}{\alpha F} \ln \frac{i}{i_0}, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

where $R$, $T$, and $F$ are the gas constant, temperature, and Faraday respectively and $\alpha$ is called the transfer coefficient and is related to the Tafel $b$ by

$$\alpha = 2 \cdot 303RT/bF; \quad \ldots\ldots\ldots\ldots\ldots \quad (3)$$

$\alpha$ may be regarded as the fraction of the potential which is favouring the forward reaction. $i_0$ is the "exchange current" which by definition is the current flowing when $\eta = 0$. At this potential the currents for the forward and backward reactions are equal.

The effect of temperature on the rate of an electrode process may be obtained in terms of $i_0$ from the absolute rate theory (Glasstone, Laidler, and Eyring 1941):

$$i_0 = KT \exp(\Delta S_0^*/R) \exp(-\Delta H_0^*/RT), \quad \ldots\ldots\ldots \quad (4)$$

or

$$\ln i_0 = \ln K + \ln T + \Delta S_0^*/R - \Delta H_0^*/RT, \quad \ldots\ldots\ldots\ldots \quad (5)$$

therefore

$$\alpha \ln i_0/\alpha\, 1/T = -T - \Delta H_0^*/R, \quad \ldots\ldots\ldots\ldots\ldots \quad (6)$$

where $K$ is a constant, $\Delta S_0^*$ is the entropy of activation, and $\Delta H_0^*$ is the heat of activation when $\eta = 0$. The value of $T$ will be small with respect to $\Delta H_0^*$ and may be neglected. The value of $\Delta H_0^*$ may also be derived directly from the effect of temperature on $\eta$ at constant $i$ by considering equations (2) and (5), and Bockris (1953) has shown that there are two cases. Firstly, when $\alpha$ is independent of temperature

$$\left(\frac{\partial \eta}{\partial T}\right)_i = \frac{\alpha\eta F + \Delta H_0^* + RT}{\alpha FT}, \quad \ldots\ldots\ldots\ldots\ldots \quad (7)$$

and secondly, when $b$ is independent of temperature

$$\left(\frac{\partial \eta}{\partial T}\right)_i = \frac{\Delta H_0^* + RT}{\alpha FT}. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (8)$$

## II. Experimental

The cell and reference electrode were similar to those used by Jones, Lind, and Wynne-Jones (1954). The cell, a modified H-cell, was supported in a water thermostat controlled at $25 \pm 0 \cdot 2$ °C. The electrode potential was measured by a Luggin capillary and the reference electrode was $Pt,PbO_2,PbSO_4/2N\ H_2SO_4$. The lead dioxide was prepared electrolytically from a solution of lead nitrate and digested in sulphuric acid at 50 °C for several days. An equal weight of recrystallized "Analar" lead sulphate was added to the dioxide and a cleaned platinum wire inserted into the paste made by mixing the solids with $2N\ H_2SO_4$. The

potential of this electrode was measured against a hydrogen electrode at 25 °C and was found to be 1·634 V. This is somewhat higher than the 1·625 V found by Hamer (1935) but since our value was stable it will be used for any conversions from the $PbSO_4/PbO_2$ to the hydrogen scale. The overpotentials are referred to the reversible oxygen potential of 1·229 V (Latimer 1938).

The electrode holders for the lead anode and platinum cathode were made of Perspex and Polythene respectively. There were no rubber connections in the cell which was made of Pyrex glass. Tap water was double distilled from alkaline permanganate before use and Analar sulphuric acid was distilled under vacuum. All solutions contained 2N $H_2SO_4$ unless otherwise stated. The lead anodes were prepared from 99·99 per cent. zone-purified lead. Strips of the metal were drawn through dies to form wires of 2 mm diameter, the ends of which were rolled flat and then cut to form an electrode surface of approximately 2 $cm^2$. They were then washed with alcohol and electrolysed anodically and cathodically in dilute perchloric acid before use.

The current was supplied by two 12 V lead accumulators through a multi-range milliammeter and controlled manually by two wire-wound potentiometers. The anode potential was measured on a Tinsley vernier potentiometer to the nearest millivolt.

## III. Results

The overpotentials ($\eta$) refer to the reversible potential of 1·229 V for the oxygen electrode (Latimer 1938). At 1 atm pressure the reversible electrode potentials for oxygen and hydrogen respectively may be written

$$E_{O_2} = 1{\cdot}229 - 0{\cdot}0591\ \mathrm{pH}, \quad \ldots\ldots\ldots\ldots\ldots \quad \textbf{(i)}$$

$$E_{H_2} = 0{\cdot}000 - 0{\cdot}0591\ \mathrm{pH}. \quad \ldots\ldots\ldots\ldots\ldots \quad \textbf{(ii)}$$

The anode potentials were measured against a $Pt,PbO_2,PbSO_4/2N\ H_2SO_4$ reference electrode which was itself calibrated against a hydrogen electrode in 2N $H_2SO_4$ so that the anode potentials refer to hydrogen in 2N $H_2SO_4$. Since all the oxygen overpotentials were measured in 2N $H_2SO_4$ the pH terms in equations **(i)** and **(ii)** will cancel out and the value of 1·229 V may be used for the reversible potential.

The effect of temperature on the overpotential ($\eta$)-log current density ($i$) curves for oxygen evolution on lead dioxide in 2N $H_2SO_4$ is shown in Figure 1. The resulting kinetic parameters appear in Table 1.

The value of $b$ appears to be independent of temperature and consequently equation **(8)** was used for the calculation of $\Delta H_0^*$. The results in Table 1 were obtained from two traverses each of decreasing and increasing current density in consecutive experiments on the same lead anode and the values of $b$ and $i_0$ are somewhat lower than the mean of eight individual experiments in which three different anodes were used. At 25 °C the following values and mean deviations were obtained $b = 0{\cdot}101 \pm 0{\cdot}002$ ; $\alpha = 0{\cdot}59 \pm 0{\cdot}01$ ; $i_0 = 7 \pm 3 \times 10^{-11}$ A $cm^{-2}$. The values of $i_0$ were determined in all cases by extrapolation to $\eta = 0$ from a current density of 0·5 mA $cm^{-2}$. At lower current densities the self-discharge of the lead dioxide surface was sufficiently rapid to give a positive deviation from the linear $\eta$-log $i$ curve.

The $\eta$-log $i$ curves of 2N $H_2SO_4$ containing 13 mg/l cobalt added as cobaltous sulphate are shown in Figure 2. The " down " curves (decreasing c.d.) and the " up " curves (increasing c.d.) differ both in $\eta$ and $b$. When 200 mg/l cobalt was used this hysteresis disappeared and it probably results from changes in the surface concentration of cobalt during the traverse from maximum to minimum

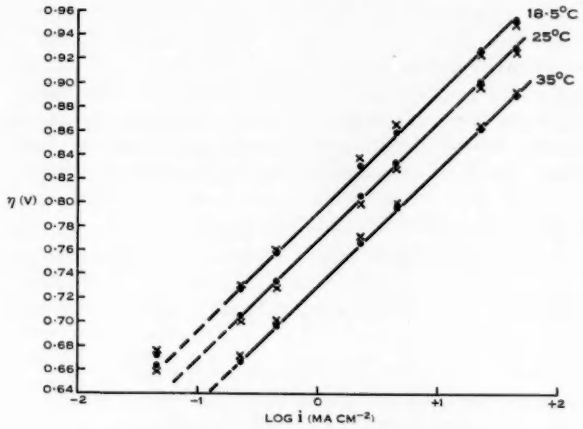


Fig. 1.—Effect of temperature on $\eta$ in 2N $H_2SO_4$.

current density. Cobalt is deposited on lead dioxide to a small extent during electrolysis (Kiseleva and Kabanov 1956) and it is possible that this reaction is partly reversible and potential dependent so that cobalt goes into solution when the potential is decreased, thus giving an increased cobalt concentration near the electrode surface. It was also found that agitation of the solution near the electrode by a stream of oxygen decreased the observed potentials, particularly

TABLE 1
EFFECT OF TEMPERATURE ON KINETIC PARAMETERS

| Temperature (°C) | $b$ | $\alpha$ | $i_0$ (A cm$^{-2}$) | $\Delta H_0^*$ (kcal mole$^{-1}$) |
|---|---|---|---|---|
| 18·5 | 0·100 | 0·58 | $1{\cdot}2\times10^{-11}$ | |
| 25·0 | 0·098 | 0·61 | $1{\cdot}6\times10^{-11}$ | 15 |
| 35·0 | 0·098 | 0·63 | $4{\cdot}0\times10^{-11}$ | |

at the lower current densities, but increasing the acid concentration to 4N $H_2SO_4$ eliminated this agitation effect. These results suggest that the difference in overpotential is due to increased cobalt concentration at the electrode surface by the solution of an oxide or hydroxide of cobalt which is soluble in acid and whose solution rate is dependent on the acid concentration at the surface as well as the potential.

The standard method for conditioning a lead dioxide anode has been a 60 min treatment at 100 mA $cm^{-2}$ and for means of comparison with the results in the absence of cobalt the down curves in Figure 2, obtained from an anode conditioned at a higher current density, have been used to calculate the kinetic parameters (Table 2).

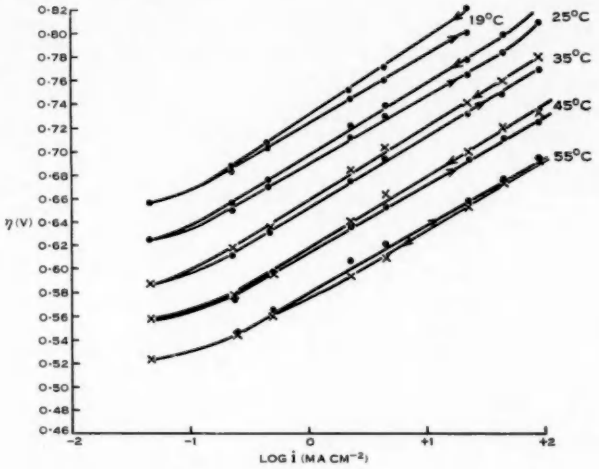


Fig. 2.—Effect of temperature on $\eta$ in 2N $H_2SO_4$+13 mg/l Co.

The values of $b$ appear independent of temperature and consequently equation (**8**) was again used to determine $\Delta H_0^{\bullet}$. A mean value of 1·03 was used for $\alpha$.

The effect of cobalt concentration at 25 °C is shown in Figure 3 and the corresponding constants in Table 3.

TABLE 2

EFFECT OF TEMPERATURE ON KINETIC PARAMETERS IN 13 MG/L Co

| Temperature (°C) | $b$ | $\alpha$ | $i_0$ (A) | $\Delta H_0^{\bullet}$ (kcal $mole^{-1}$) |
|---|---|---|---|---|
| 25 | 0·061 | 0·98 | $4\times10^{-15}$ | |
| 35 | 0·061 | 1·01 | $2\times10^{-14}$ | 29 |
| 45 | 0·061 | 1·05 | $8\times10^{-14}$ | |
| 55 | 0·060 | 1·09 | $3\times10^{-13}$ | |

In order to determine the effect of the anode material on the overpotential the $\eta$-log $i$ curves for oxygen evolution on bright platinum in 2N $H_2SO_4$ with and without cobalt were studied (Fig. 4). It is evident that cobalt has no significant effect on the oxygen evolution at a platinum anode.

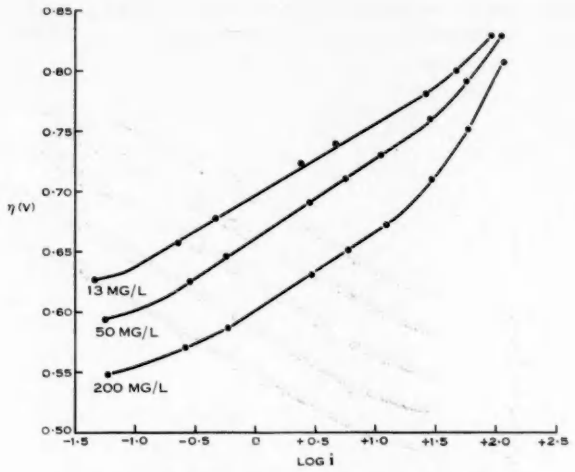


Fig. 3.—Effect of [$Co^{++}$] on $\eta$ in 2N $H_2SO_4$ at 25 °C.

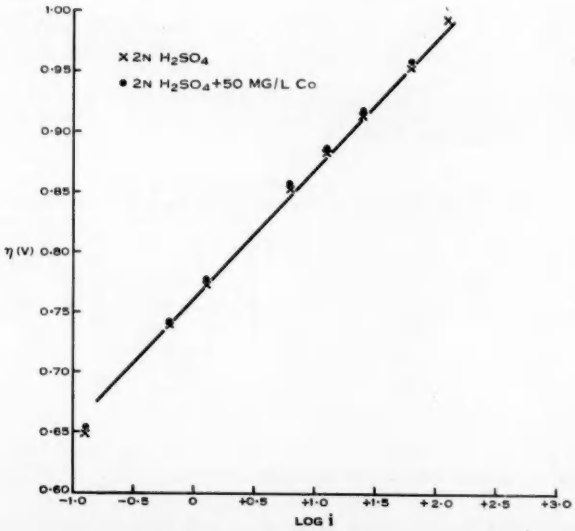


Fig. 4.—Effect of Co on $\eta$ at a platinum anode at 25 °C.

## IV. Discussion

The values of $\alpha$ obtained for oxygen evolution on lead dioxide both in the presence and absence of cobalt appear temperature dependent, while Bowden (1929) and Turyan and Gershkovich (1956) have found $\alpha$ independent of temperature for oxygen evolution on platinum and cobalt respectively. Bockris and Parsons (1949) have obtained a temperature dependent $\alpha$ for hydrogen evolution on mercury and point out that the temperature dependence of double layer thickness, adsorption and solvation energies of the ions could be reflected in the value $\alpha$. It is possible that the effect in the present case is a temperature dependent adsorption energy characteristic of the lead dioxide surface since neither cobalt nor platinum anodes give a temperature dependent $\alpha$.

Table 3

Effect of [Co] on kinetic parameters at 25 °C

| [Co] (mg/l) | $b$ | $\alpha$ | $i_0$ (A) |
|---|---|---|---|
| 13 | 0·061 | 0·98 | $4\times10^{-15}$ |
| 50 | 0·065 | 0·92 | $6\times10^{-14}$ |
| 200 | 0·067 | 0·89 | $1\times10^{-12}$ |

In the absence of cobalt the values of $\alpha$ are in agreement with the mechanism suggested by Bockris (1956) viz.:

$$OH^- + M \rightleftharpoons MOH + e, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (9)$$

$$2MOH \rightleftharpoons MO + M + H_2O, \quad \ldots\ldots\ldots\ldots \quad (10)$$

$$2MO \rightleftharpoons 2M + O_2, \quad \ldots\ldots\ldots\ldots\ldots \quad (11)$$

where the discharge of $OH^-$ (or $H_2O$ in acid solution) is the rate determining step. M refers to the lead dioxide surface. Evidence for rate control by equation (9) for the evolution of oxygen on platinum has been put forward by Bockris and Shamshul Huq (1956). The present value of 15 kcal mole$^{-1}$ for $\Delta H_0^*$ is not very different from the 19 kcal mole$^{-1}$ obtained by Bowden (1929) for oxygen evolution on platinum and it is therefore very likely that discharge of $OH^-$ or water molecules on lead oxide is the rate determining step in the absence of cobalt.

When cobalt is added to the solution the overpotential falls sharply and the values of $\alpha$ are now nearer 1·0 than 0·5. Furthermore $\Delta H_0^*$ has risen to 29 kcal mole$^{-1}$ suggesting that a different mechanism is rate determining. $\alpha$ values of 1·0 have also been observed by Turyan and Gershkovich (1956) for oxygen evolution at cobalt anodes in alkaline solution and they suggest that a possible rate determining step in alkaline solution is

$$2Co_2O_4 + 6H_2O \rightarrow Co(OH)_3 + O_2. \quad \ldots\ldots\ldots\ldots \quad (12)$$

The potentials at the lead dioxide anode in all cases are more positive than the reversible potential of 1·84 at 25 °C (Latimer 1938) for the couple

$Co^{++} \rightleftharpoons Co^{+++} + e$ so that cobaltic ions can be formed at the anode. The evidence for this reaction being part of the mechanism will be discussed below but it is worth noting that cobalt had no effect on the oxygen evolution at a bright platinum anode (Fig. 4) so that it is likely that the oxidation of cobaltous to cobaltic is catalysed by the lead dioxide surface. The small deposition of cobalt in the lead dioxide is evidence of a cobalt-lead dioxide bonding.

A rate determining discharge such as equation (**9**) will not give a Tafel $b$ value of 0·058 and it is necessary to postulate one of the succeeding steps as rate determining. The following mechanism is suggested where either steps 14 or 15 is rate determining.

$$Co^{++} \underset{v_{-1}}{\overset{v_1}{\rightleftharpoons}} Co^{+++} + e, \quad \ldots\ldots\ldots\ldots\ldots \quad (13)$$

$$Co^{+++} + OH^- \underset{v_{-2}}{\overset{v_2}{\rightleftharpoons}} CoOH^{++}, \quad \ldots\ldots\ldots\ldots\ldots \quad (14)$$

$$2CoOH^{++} \underset{v_{-3}}{\overset{v_3}{\rightleftharpoons}} 2Co^{++} + H_2O + O, \quad \ldots\ldots\ldots \quad (15)$$

$$O + O \underset{v_{-4}}{\overset{v_4}{\rightleftharpoons}} O_2. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (16)$$

Discharge of a water molecule is more likely than $OH^-$ in acid solution and this may be substituted for hydroxyl ion without affecting the argument. $CoOH^{++}$ has been suggested as the simplest complex. Either $Co(OH)_2^+$ or $Co(OH)_3$ will give the same final dependence of $\eta_i$ on log $i$ and log $(Co^{++})$. Step (**13**) is catalysed by lead dioxide and the resulting $Co^{+++}$ is adsorbed on the surface where it combines with a hydroxyl ion or water molecule to produce a complex.

Bockris (1956) has shown how the overall rate equation for a series of consecutive reactions may be obtained from the rates of the forward and backward reactions of each step by application of an equation derived by Christiansen (1936). For $n$ consecutive steps in a reaction the overall rate may be expressed by

$$\frac{1}{v} = \frac{1}{w_1} + \frac{w_{-1}}{w_1 w_2} + \frac{w_{-1}w_{-2}}{w_1 w_2 w_3} + \ldots + \frac{w_{-1}w_{-2}\ldots w_{-(n-1)}}{w_1 w_2 w_3 \ldots w_n}, \quad \ldots\ldots \quad (17)$$

where $v$ is the velocity of the reactions and $w_1, w_2, \ldots, w_n$ are defined by

$$\begin{aligned} w_1 &= v_1, & w_{-1} &= v_{-1}/c_1, \\ w_2 &= v_2/c_1, & w_{-2} &= v_{-2}/c_2, \\ w_3 &= v_3/c_2 \ldots, & & \\ w_n &= v_n/c_{n-1}, & w_{-n} &= v_{-n}/c_n, \end{aligned}$$

where $v_n$ is the velocity of the forward and $v_{-n}$ the backward reactions of the $n$th step. $c_1, c_2, \ldots, c_n$ are the concentrations of the entities produced by steps 1, 2, . . ., $n$.

The rate of an anodic process may be expressed as

$$v = k \exp(\beta n \eta F/RT), \quad \ldots\ldots\ldots\ldots\ldots \quad (18)$$

where $\beta$ is the symmetry factor and will be taken as 0·5 for reaction (**13**), $n$ is the number of electrons taking part in the reaction, and $k$ is the rate constant. A series of equations expressing the forward ($v_n$) and backward ($v_{-n}$) rates may now be written for the reaction series in equations (**13**), (**14**), and (**15**). Assuming that the final step (**16**), the combination of oxygen atoms, is fast and may be neglected. Let $x$ equal the fraction of the effective area covered by $Co^{+++}$, then $1-x$ equals the fraction covered by $CoOH^{++}$.

$$v_1=k_1(Co^{++})\exp(\eta_1 F/2RT), \quad \ldots\ldots\ldots\ldots (19)$$
$$v_{-1}=k_{-1}x\exp(-\eta_1 F/2RT), \quad \ldots\ldots\ldots\ldots (20)$$
$$v_2=k_2 x(OH^-), \quad \ldots\ldots\ldots\ldots (21)$$
$$v_{-2}=k_{-2}(1-x), \quad \ldots\ldots\ldots\ldots (22)$$
$$v_3=k_3(1-x)^2, \quad \ldots\ldots\ldots\ldots (23)$$
$$v_{-3}=k_{-3}(Co^{++})^2 PO_2^{\frac{1}{2}}. \quad \ldots\ldots\ldots\ldots (24)$$

Expressing these values of $v_n$ in terms of $w_n$

$$w_1=v_1=k_1(Co^{++})\exp(\eta_1 F/2RT), \quad \ldots\ldots\ldots\ldots (25)$$
$$w_{-1}=v_{-1}/x=k_{-1}\exp(-\eta_1 F/2RT), \quad \ldots\ldots\ldots\ldots (26)$$
$$w_2=v_2/x=k_2(OH^-), \quad \ldots\ldots\ldots\ldots (27)$$
$$w_{-2}=v_{-2}/(1-x)=k_{-2}, \quad \ldots\ldots\ldots\ldots (28)$$
$$w_3=v_3/(1-x)=k_3(1-x), \quad \ldots\ldots\ldots\ldots (29)$$
$$w_{-3}=v_{-3}/(Co^{++})=k_{-3}(Co^{++}) \quad \text{(if } PO_2 \text{ is constant).} \quad \ldots\ldots (30)$$

Applying equation (**17**) to this series of equations

$$\frac{1}{v}=\frac{1}{k_1(Co^{++})\exp(\eta_1 F/2RT)}+\frac{k_1\exp(-\eta_1 F/2RT)}{k_1(Co^{++})\exp(\eta_1 F/2RT)k_2(OH^-)}$$
$$+\frac{k_{-1}\exp(-\eta_1 F/2RT)k_{-2}}{k_1(Co^{++})\exp(\eta_1 F/2RT)\,.\,k_2(OH^-)\,.\,k_3(1-x)} \quad \ldots\ldots\ldots\ldots (31)$$

$$=\frac{k_2k_3(OH^-)(1-x)+k_{-1}k_3(1-x)\exp(-\eta_1 F/2RT)+k_{-1}k_{-2}\exp(-\eta_1 F/2RT)}{k_1k_2k_3(Co^{++})(OH^-)(1-x)\exp(\eta_1 F/2RT)}. \quad \ldots\ldots\ldots\ldots (32)$$

If it is now assumed that reaction (**15**) is the slow step so that concentration of $Co^{+++}$ on the surface is negligible then $x\to 0$ and

$$v=\frac{k_1k_2k_3(Co^{++})(OH^-)\exp(\eta_1 F/2RT)}{k_2k_3(OH^-)+k_{-1}k_3\exp(-\eta_1 F/2RT)+k_{-1}k_{-2}\exp(-\eta_1 F/2RT)}, \quad \ldots (33)$$

with $k_3\to k_{-3}\to 0$ terms in the denominator with $k_3$ and $k_{-3}$ may be neglected. Thus

$$v=\frac{k_1k_2k_3}{k_{-1}k_{-2}}(Co^{++})(OH^-)\exp(\eta_1 F/RT), \quad \ldots\ldots\ldots (34)$$

$$i=F\frac{k_1k_2k_3}{k_{-1}k_{-2}}(Co^{++})(OH^-)\exp(\eta_1 F/RT), \quad \ldots\ldots\ldots (35)$$

therefore,

$$\left(\frac{\partial \eta}{\partial \ln i}\right)_{Co^{++},\,OH^-} = \frac{RT}{F}, \quad \ldots\ldots\ldots\ldots\ldots \quad (36)$$

that is, $b = 0{\cdot}060$ at 25 °C which was found experimentally. Furthermore equation (**35**) shows that at constant current

$$\left(\frac{\partial \eta}{\partial \ln (Co^{++})}\right)_{i,\,OH^-} = \frac{RT}{F}, \quad \ldots\ldots\ldots\ldots\ldots \quad (37)$$

and a plot of $\eta$ *v.* log [$Co^{++}$] in Figure 5 gives a gradient of 0·060 in agreement with that predicted by equation (**37**).

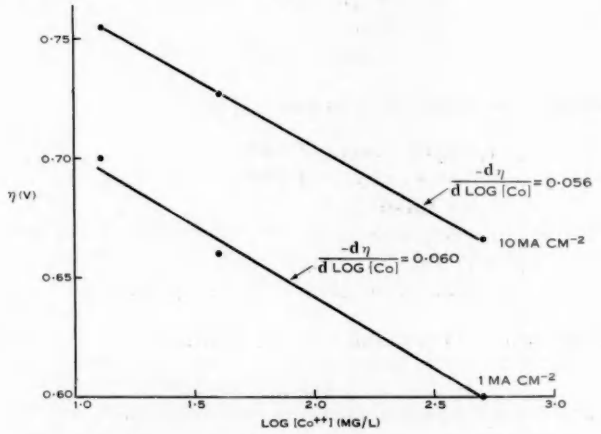


**Fig. 5.**—Effect of [$Co^{++}$] on $\eta$ in 2N $H_2SO_4$ at two current densities, temperature 25 °C.

The same values for $\partial\eta/\partial \ln i$ and $\partial\eta/\partial \ln [Co^{++}]$ are obtained by the above treatment if the preceding step, reaction (**14**) is rate determining. In this case, since the steps after the one which is rate determining may be neglected,

$$\frac{1}{v} = \frac{1}{k_1(Co^{++}) \exp(\eta F/2RT)} + \frac{k_{-1} \exp(-\eta F/2RT)}{k_1(Co^{++}) \exp(\eta F/2RT) k_2(OH^-)}$$

$$= \frac{k_2(OH^-) + k_{-1} \exp(-\eta F/2RT)}{k_1 k_2 (Co^{++})(OH^-) \exp(\eta F/2RT)}, \quad \ldots\ldots\ldots\ldots\ldots \quad (38)$$

$$v = \frac{k_1 k_2 (Co^{++})(OH^-) \exp(\eta F/2RT)}{k_2(OH^-) + k_{-1} \exp(-\eta F/2RT)}, \quad \ldots\ldots\ldots\ldots\ldots \quad (39)$$

and if $k_2 \to 0$

$$v = \frac{k_1 k_2}{k_{-1}} (Co^{++})(OH^-) \exp(\eta F/RT). \quad \ldots\ldots\ldots\ldots\ldots \quad (40)$$

Using the relationship $k_n/k_{-n}=K_n$, where $K_n$ is the equilibrium constant for the $n$th reaction, equation (**34**) may be written

$$v=K_1K_2k_3(\mathrm{Co}^{++})(\mathrm{OH}^-)\exp(\eta F/RT), \quad \ldots\ldots\ (41)$$

and equation (**40**) as

$$v=K_1k_2(\mathrm{Co}^{++})(\mathrm{OH}^-)\exp(\eta F/RT). \quad \ldots\ldots\ (42)$$

This gives

$$k_2/k_3=K_2,$$

and if $K_2\gg 1$, $k_3$ will be the rate determining step. The equilibrium constant for the reaction

$$\mathrm{Co}^{+++}+3\mathrm{OH}^-=\mathrm{Co(OH)_3}$$

is $10^{43}$ (Latimer 1938 ; Sobol 1953) and if $K_2$ is of the same order $k_3$ will be rate determining, that is, the slow step in the reaction will be

$$2\mathrm{CoOH}^{++}\rightleftharpoons 2\mathrm{Co}^{++}+\mathrm{H_2O}+\mathrm{O}.$$

## V. Acknowledgments

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# DECOMPOSITION OF HYDROGEN PEROXIDE CATALYSED BY RUTHENIUM COMPLEXES

By F. P. Dwyer,* N. Kelso King,† and M. E. Winfield†

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*Summary*

A preliminary study has been made of the oxidation of four octahedral $Ru^{II}$ complexes in aqueous solution, particularly by $H_2O_2$. Kinetic measurements and simple experiments such as electrolytic oxidation followed by reduction with $H_2O_2$ have been used to obtain evidence for the mechanism of $H_2O_2$ decomposition when catalysed by the ruthenium complexes. The main course of the catalysis appears to consist in oxidation of $Ru^{II}$ to $Ru^{IV}$ with a corresponding reduction of $H_2O_2$ to hydroxyl ions, followed by a 2-electron reduction of $Ru^{IV}$ to $Ru^{II}$ with oxidation of $H_2O_2$ to $O_2$ and hydrogen ions.

## I. Introduction

There are thermodynamic grounds for believing that compounds may exist which can catalyse the decomposition of $H_2O_2$ by a cyclic process in which the catalyst suffers first a 2-electron oxidation then a 2-electron reduction, with little or no formation of radicals such as OH or OOH (King and Winfield 1959*a*, 1959*b*). It was suggested that such a catalyst would be a metal complex for which $E^0 = -0{\cdot}8$ V (approximately) in the reaction

$$M^{n+} \rightarrow M^{(n+2)+} + 2e.$$

Also that in the decomposition of $H_2O_2$ in the presence of catalase there are possibly two kinds of reactive site on the enzyme, one a metal atom and the other a carbon atom of one of the ligands. We have therefore tried to select metal complexes with the desired properties, and have examined for catalytic activity a series of four $Ru^{II}$ compounds synthesized by Dwyer and Goodwin (unpublished data). These are 6-coordinate complexes in which the ligands are strongly held, as judged from the stability in strong acid or alkali. The $E^0$ values are more negative than desired and it is hoped later to synthesize complexes with ideal oxidation potentials. However, the four selected (I, II, III, IV) were all oxidizable at least in part to the $Ru^{IV}$ state by $H_2O_2$.

| | |
|---|---|
| $[Ru^{II}\ dipy_2\ gly](ClO_4)$ (I) | $[Ru^{II}\ acac\ dipy_2](ClO_4)$ (II) |
| $[Ru^{II}\ gly\ py_4](ClO_4)$ (III) | $[Ru^{II}\ acac\ py_4](ClO_4)$ (IV) |

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(Dipy, gly, acac, and py represent dipyridyl, glycinato, acetylacetonato, and pyridine groups respectively.) Since they were 6-covalent it seemed possible that a weak coordinating agent such as $\bar{O}OH$ would oxidize an existing ligand rather than add to the metal in a seventh position.

## II. Kinetics

(*a*) *Oxidation of Ligands.*—The enzyme catalase is slowly destroyed by $H_2O_2$, probably due to irreversible oxidation of one of the ligands. The ruthenium complexes (I–IV) proved to be much more resistant, but only I resisted irreversible oxidation for long periods. Figure 1 shows the amount of gas liberated from

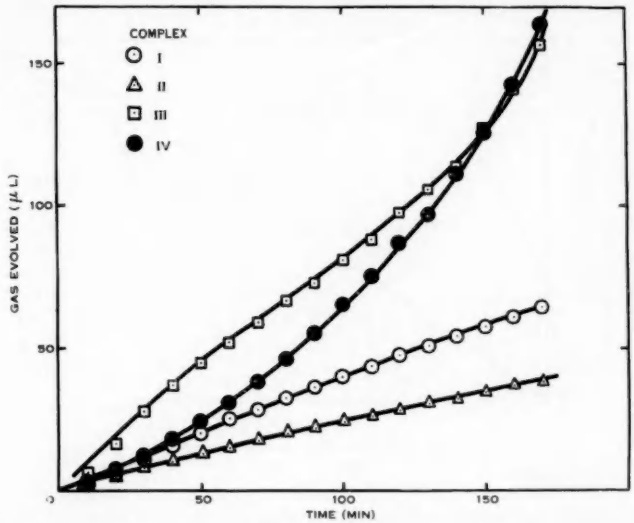


Fig. 1.—Oxygen evolution from $H_2O_2$ as a function of time, in the presence of ruthenium complexes I, II, III, and IV.
In argon at 30 °C; 3 ml of reaction mixture; $H_2O_2$ 0·02M; complexes I and II 0·00025M; complexes III and IV 0·00001M; pH 7·0 phosphate buffer 0·1M.

$H_2O_2$, by each of the complexes, as a function of time. For I the rate of gas evolution was nearly constant when corrected for decline in $H_2O_2$ concentration. Later it was shown to be essentially constant for 24 hr at 30 °C at an $H_2O_2$ concentration of $2 \times 10^{-3}$M. For complexes III and IV the rate was much higher and increased markedly with time. It was concluded that one of the chelated groups was slowly oxidized or detached to form a complex of much greater catalytic activity.

(*b*) *Dependence of Rate on Ruthenium Concentration.*—When steady-state rates (after 50 min) were plotted as a function of catalyst concentration (complex I), they were found to be proportional to the first power of the total ruthenium concentration, irrespective of oxidation state.

(*c*) *Dependence of Rate on $H_2O_2$ Concentration.*—A straight line was not obtained on plotting steady-state rates at pH 7·2 against the first power of the $H_2O_2$ concentration (complex I). Figure 2 shows that the rates were proportional to the initial peroxide concentration raised to the power 1·2. The $H_2O_2$ concentration was sufficiently high to ensure negligible decrease during the course of the experiment.

A linear relation between rates and peroxide concentration was obtained at pH 4·5.

(*d*) *Dependence of Rate on pH.*—The reaction mixture was buffered, usually with potassium phosphate at a molarity of 0·1M. Before and after rate measurements the pH was measured and shown to have undergone little change. Below

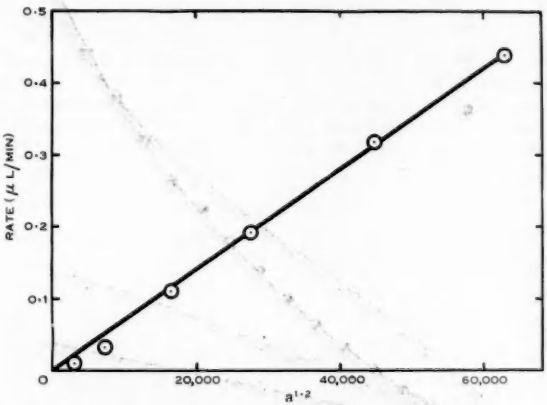


Fig. 2.—Dependence of rate of $O_2$ evolution on peroxide concentration.
In argon at 30 °C ; 3 ml of reaction mixture ; complex I 0·0005M ; pH 7·2 phosphate buffer 0·1M ; *a*, initial $H_2O_2$ concentration in μmole $l^{-1}$.

pH 5·5 it was necessary to use acetate buffer in place of phosphate and tris-(hydroxymethyl)aminomethane buffer above pH 8·0. Changing the nature of the buffer was shown not to influence the reaction rate except in so far as the pH was changed.

For complex I, optima were found at about pH 5 and 7. Figure 3 shows the rates measured 50 min after addition of catalyst to the buffered $H_2O_2$ solution. Whereas at pH 7 the rate of gas evolution continued steadily for long periods, a marked decline with time was noted at pH 5 and the decline was accompanied by formation of a green $Ru^{III}$ complex (see Section III (*b*)).

With complex II, as catalyst optima occurred at pH 3·5 and 6·8 ; with complex IV at pH 4·8 and 7·4.

(*e*) *Inhibition by Enzyme Poisons.*—It was expected that strongly coordinating groups such as $\bar{C}N$ would at low concentration prevent attachment of $\bar{O}OH$ to the metal, yet fail to attack or displace ligands which were already coordinated.

Figure 4 shows that the activity of complex I was depressed only weakly by KCN at the concentrations used in enzyme inhibition. At low concentration, $NH_2OH$ was also inhibitory. The apparent stimulation in the presence of 0·001M $NH_2OH$ was shown to be the result of a reaction between $NH_2OH$ and $H_2O_2$. Nearly one-half of the gas evolved could not be absorbed in alkaline pyrogallol or aqueous KOH, and therefore was taken to be $N_2$. At the lower $NH_2OH$ concentration, and also in the presence of KCN, the yield of gas which could not be absorbed in an aqueous mixture of pyrogallol and $K_2CO_3$ was negligible.

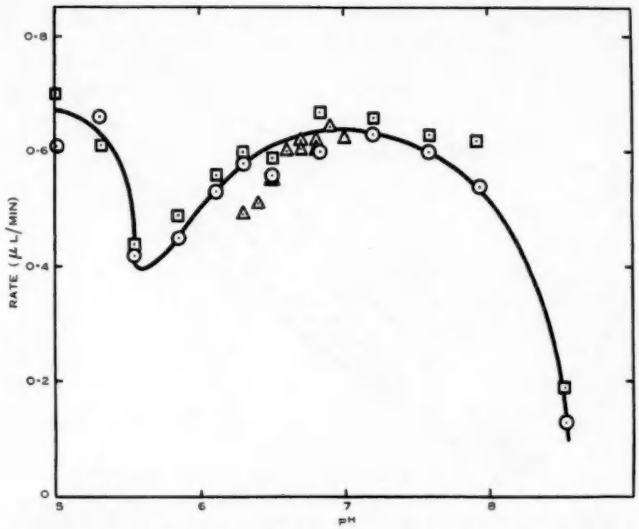


Fig. 3.—Optimum pH for peroxide decomposition catalysed by complex I. In argon at 30 °C; 3 ml of reaction mixture; $H_2O_2$ 0·01M; complex I 0·0005M; buffer 0·1M.

In Figure 5 the curves refer to complex IV, which as we have already seen is slowly converted by $H_2O_2$ to a much more active catalyst. In amounts equimolar with IV, KCN was inhibitory but shortened the induction period. At 10 times higher concentration it lengthened the induction period by a factor of 4 to 5, but there was no inhibition of the maximum rate of gas evolution.

Hydroxylamine at the lower concentration (0·0001M) gave a weak stimulation. Less than 2 per cent. of the gas evolved was $N_2$. At the higher concentration it increased the induction period by a factor of 5, and inhibited $O_2$ evolution about 50 per cent. (taking into account the amount of $N_2$ evolved).

$NH_2OH$ is a good reducing agent, and it is shown in Section III (*b*) that addition of KCN to oxidized ruthenium complex can also result in reduction. Both inhibitors therefore tend to delay the establishment of the steady state in the experiments of Figures 4 and 5. On the other hand, they probably hasten

conversion of IV to the modified complex which exhibits enhanced catalytic activity.

The weak cyanide inhibition suggests that $\bar{C}N$ can add to the ruthenium atom in a seventh position but the equilibrium concentration of the product is small. With $\bar{O}OH$ as seventh ligand, it is likely to be much smaller.

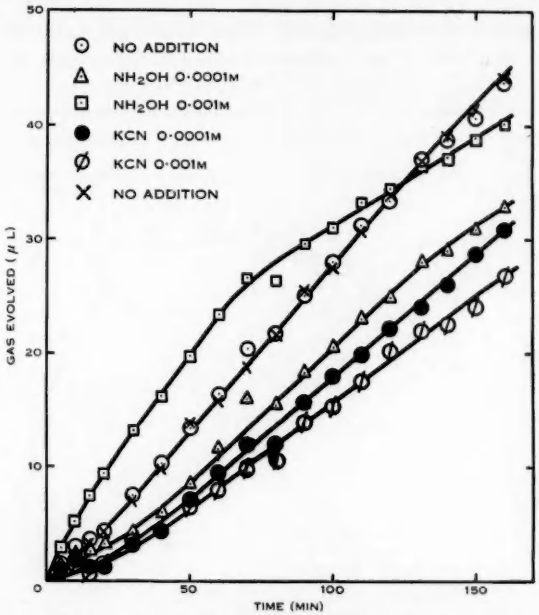


Fig. 4.—Inhibition by KCN and $NH_2OH$ of catalysis by complex I. In argon at 30 °C; 3 ml of reaction mixture; $H_2O_2$ 0·01M; catalyst 0·0005M; pH 7·4 phosphate buffer 0·1M. Experiments with no added inhibitor were carried out at the beginning and end of the series, so that their degree of correspondence provided a check on possible deviations in temperature, timing, cell calibration, etc.

## III. Electrolytic Oxidation and Reduction

### (*a*) *Oxidation*

In order to oxidize a large proportion of the red $Ru^{II}$ beyond the blue $Ru^{III}$ state (see Section III (*c*) for relation between colour and oxidation state), it was necessary to work at acid pH. The experiments were usually carried out in 1·5M acetate buffer of pH 3·5, with complex II at a concentration of $7 \times 10^{-4}$M. At the anode, the pH fell to approximately 2 in the course of an experiment. The pale blue colour of the $Ru^{III}$ form was not always observed, since only small amounts of the more intensely coloured $Ru^{II}$ and $Ru^{IV}$ were sufficient to obscure it.

In a typical experiment, 2 ml of a solution of II was oxidized from red ($Ru^{II}$) to pale brown, and as soon as the latter was largely oxidized to the red

$Ru^{IV}$ state a 0·5 ml sample was withdrawn. To this was added one drop of 30 per cent. hydrogen peroxide solution (A.R. grade). There was an immediate reduction of the complex to an oxidation state corresponding to a mixture of $Ru^{II}$ and $Ru^{III}$, and a brief evolution of gas.* Thirty seconds later a slow but continuous evolution of gas commenced.

After a further short period of electrolytic oxidation, some of the red $Ru^{IV}$ complex had been converted to a pink modification. On addition of $H_2O_2$ there was immediate reduction to a modified $Ru^{II}$ or $Ru^{III}$ complex (yellow) and a brief evolution of gas. After 5 sec continuous evolution commenced and proceeded at a rate several times faster than for the first sample withdrawn.

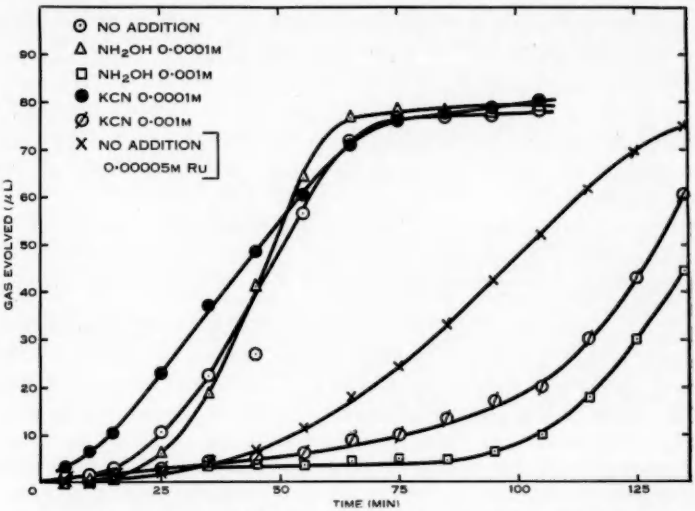


Fig. 5.—Inhibition by KCN and $NH_2OH$ of catalysis by complex IV.
In argon at 30 °C; 3 ml reaction mixture; $H_2O_2$ 0·0025M; catalyst 0·0001M; pH 7·4 phosphate buffer 0·1M.

We interpret these experiments, in conjunction with the results of Figure 1, as follows:

In its doubly oxidized form, II reacts directly and rapidly with $H_2O_2$ to yield $O_2$ and the reduced ($Ru^{II}$) complex. The latter is then slowly oxidized by $H_2O_2$ and a steady state is established in which the concentration of $Ru^{II}$ is much greater than that of $Ru^{IV}$. When the $Ru^{IV}$ compound is subjected to a strongly oxidizing environment, it is slowly converted to a pink complex in which a ligand has been oxidized or detached. The change in structure enhances considerably the catalytic activity.

* Only with the doubly oxidized ruthenium complex could we observe an immediate gas evolution. The $Ru^{III}$ state referred to here is the blue rather than the green modification mentioned in the next section.

### (*b*) *Reduction*

The red $Ru^{IV}$ was rapidly reduced to a green complex, believed to contain $Ru^{III}$ (Section III (*a*)). After prolonged electrolytic reduction the latter was converted to the original $Ru^{II}$ complex (II). By contrast the blue $Ru^{III}$ (formed by gentle oxidation of II) was readily reduced to the $Ru^{II}$ state. In spite of the acid pH, which favours formation of the green $Ru^{III}$ compound, the green colour did not appear when $H_2O_2$ was used to reduce red $Ru^{IV}$.

Ascorbic acid reduced the red $Ru^{IV}$ complex immediately by a 1-electron step to green $Ru^{III}$ and then slowly removed a second electron. With ethanol the reaction was slower and there was little or no reduction beyond the $Ru^{III}$ state.

Addition of KCN to a solution containing blue $Ru^{III}$ caused reduction to $Ru^{II}$. Reduction of red $Ru^{IV}$ resulting from addition of KCN yielded the green $Ru^{III}$ complex but would proceed no further.

### (*c*) *Titration*

We have not proved conclusively that the complexes have the oxidation states assigned to them on the basis of colour, but the following evidence is strongly suggestive:

Titration of II against ceric ion showed that no more than 1-electron was required to oxidize it to the blue complex. Dwyer and Goodwin (unpublished data) have shown by magnetic measurements that the blue ruthenium complexes are in the $Ru^{III}$ state.

The electrolytic reduction of red oxidized complex to green indicated that the colour change was due to addition of an electron rather than a ligand. Titration against thiosulphate ion showed that 1 or less electrons were required.

Conversion of the green compound to red $Ru^{II}$ proceeded sluggishly and required 1·5 equivalents of alkaline thiosulphate, the apparent need for more than 1-electron being due to the slowness and inefficiency of the reduction.

Complex II could not be further reduced electrolytically.

### (*d*) *Other Complexes*

Experiments of the kind described for II in Sections III (*a*), (*b*), and (*c*) were impracticable for the other complexes either because of inconvenient colours or redox potentials. Complex IV was almost as readily oxidized as II but the colours were less intense. Yellow $Ru^{II}$ was oxidized to blue $Ru^{III}$ to yellow $Ru^{IV}$. By electrolytic reduction the last was readily converted to a purple complex, presumably $Ru^{III}$, which was slow to undergo further reduction. Titration with thiosulphate ion showed that no more than 1-electron was required for the first step and at least 1-electron for the second.

## IV. Liesegang Experiments

It was possible to confirm some of the observations and conclusions of Section III by simple experiments in which a layer of 30 per cent. $H_2O_2$ was carefully introduced below 0·5 ml of a $7 \times 10^{-4}$M solution of II in 0·3M acetate buffer (pH 3·5) contained in a 7 mm test tube. Several of the coloured bands which formed could be ascribed to definite oxidation states, while others appeared

to be mixtures whose content could be deduced from the electrolytic oxidations of Section III.

By many repetitions of the experiments we were able to observe the following:

Below the red $Ru^{II}$ a narrow band of blue $Ru^{III}$ appeared, immediately above a more diffuse band of brown which was assumed to contain a mixture of red $Ru^{IV}$ and blue $Ru^{III}$. Gas evolution commenced when the brown band first became readily visible, and the bubbles originated at the top of the brown band. As a result of the gas-producing reaction, a green band appeared immediately above the brown, and shortly afterwards a narrower red band above the green. In some experiments, this narrow band was brown rather than red, due to mixing with the blue layer above.

We consider that the green ($Ru^{III}$) complex was formed by interaction of $Ru^{IV}$ (visible below the green band) with $Ru^{II}$ (visible above the green band). The fact that the rate of gas evolution declined as the concentration of green $Ru^{III}$ increased (Section II (*d*)) discounts the importance of the green complex in the catalysis. No gas evolution could be observed from the blue $Ru^{III}$ band in any of the experiments.

Oxidation of II by chlorite ion or $PbO_2$ resembled oxidation by $H_2O_2$, while ceric ion failed to produce any green $Ru^{III}$.

We have interpreted the above results to indicate that $H_2O_2$ reduces the $Ru^{IV}$ complex directly to the $Ru^{II}$ state, and that the latter is possibly oxidized by $H_2O_2$ in two 1-electron steps to $Ru^{IV}$ rather than directly. It must be remembered, however, that as soon as $Ru^{IV}$ is formed it will react with adjacent $Ru^{II}$ to yield $Ru^{III}$, so that direct 2-electron oxidation of $Ru^{II}$ to $Ru^{IV}$ cannot be discounted. In the second half of the catalytic cycle $H_2O_2$ reduces $Ru^{IV}$ to $Ru^{II}$ with liberation of $O_2$ and without formation of apparent intermediates.

## V. Discussion

On the whole, the experiments which have been described are in harmony with the simple mechanism.

$$Ru^{II}+H_2O_2 \rightarrow Ru^{IV}+2\bar{O}H, \quad \ldots\ldots\ldots\ldots\ldots \quad (1)$$

$$Ru^{IV}+H_2O_2 \rightarrow Ru^{II}+O_2+2H^+, \quad \ldots\ldots\ldots\ldots \quad (2)$$

in which $Ru^{II}$ represents the ruthenium complex.

Whether or not the reaction

$$Ru^{II}+H_2O_2 \rightarrow Ru^{III}\,(\text{blue})+\bar{O}H+\dot{O}H \quad \ldots\ldots\ldots \quad (3)$$

occurs, is obscured by the formation of $Ru^{III}$ which will result when $Ru^{IV}$ is produced in the presence of $Ru^{II}$. Thus, our observations do not lead to a definite decision on whether reaction (**1**) proceeds by two 1-electron bimolecular steps.

Since reduction by $H_2O_2$ of $Ru^{IV}$ containing no $Ru^{II}$ (Section III (*a*)) produces no green $Ru^{III}$, while electrolytic reduction of $Ru^{IV}$ or the action of $H_2O_2$ on $Ru^{II}$ (Section IV) produces a considerable amount, we believe that reaction (**2**) proceeds by way of a 2-electron reduction.

To a lesser extent than reactions (**1**), (**2**) the more familiar radical chain mechanism necessitated by catalysts which can undergo only 1-electron oxidation

may apply also, especially at the higher pH values, where the 2-electron oxidation is more difficult. By analogy with the mechanism proposed for ferrous ion (see for example, Weiss 1952), we would have chain initiating reactions such as (3) and (4):

$$Ru^{IV} + H_2O_2 \rightarrow Ru^{III}\ (green) + \dot{O}OH + H^+. \quad \ldots\ldots\ (4)$$

As the pH is diminished, the ruthenium complex becomes more readily oxidized to $Ru^{IV}$ and the importance of radical formation recedes. However, at acid pH enough $Ru^{IV}$ is present during $H_2O_2$ decomposition to permit a noticeable amount of reduction of $Ru^{IV}$ to green $Ru^{III}$ and a corresponding loss of activity. Thus a possible interpretation of the two pH optima, and the non-linear dependence of rate on $H_2O_2$ concentration at the higher pH, can be given as follows:

The optimum near pH 7 is due to the need to liberate both hydroxyl ions and hydrogen ions during the catalytic cycle. At this pH, however, little of the complex can be oxidized to $Ru^{IV}$ and so an appreciable part of the $H_2O_2$ decomposition follows the radical chain mechanism. The optimum at pH 5 is due to a balance between the need for a pH near 7, and the beneficial effect of low pH in easing the oxidation to $Ru^{IV}$, which permits almost all the catalysis to proceed by the ionic cycle.

In preparing better catalysts for the ionic mechanism, we have to aim at complexes which are easier to oxidize to $Ru^{IV}$, and at the same time we have to find a means of preventing electron transfer between $Ru^{II}$ and $Ru^{IV}$. The latter objective might be achieved with a complex which is outer orbital in the $Ru^{II}$ and inner orbital in the $Ru^{IV}$ state.*

The possibility of two types of site for $H_2O_2$ attack on the complex during the catalytic cycle has been discussed by King and Winfield (1959*a*).

With reference to the 1-electron oxidation of ascorbic acid by $Ru^{IV}$ complexes, in contrast to the 2-electron oxidation of $H_2O_2$, it is interesting to note that the enzyme peroxidase is believed to oxidize ascorbic acid in two 1-electron steps (Chance 1949; George 1953). The reduction of $Ru^{IV}$ by ascorbic acid to the inactive green $Ru^{III}$ complex is reminiscent of the irreversible destruction of catalase by ascorbic acid in the presence of $H_2O_2$ (Lemberg and Legge 1949).

## VI. Acknowledgments

The authors wish to express their thanks to Mr. J. Bayston, who carried out the kinetic measurements.

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* An analogous situation holds for catalase if our interpretation of the magnetic evidence is correct (King and Winfield 1959*a*).

# CATALYSIS OF HYDROGEN PEROXIDE DECOMPOSITION BY 2-ELECTRON OXIDATION AND REDUCTION

By N. Kelso King* and M. E. Winfield*

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*Summary*

A thermodynamical argument is used to support the suggestion made elsewhere that the more common radical chain mechanism for catalysed decomposition of $H_2O_2$ need not predominate if the catalyst can readily undergo a reversible 2-electron oxidation. How complete the exclusion of free radical formation may be depends upon the redox characteristics of the catalyst and on whether its oxidation by two single-electron steps is readily reversible along the same path.

## I. Introduction

A considerable number of compounds is now known to catalyze the decomposition of $H_2O_2$ by undergoing alternate oxidation and reduction, and it is generally supposed that in the reaction of the reduced catalyst with $H_2O_2$ there is produced a free radical, probably OH (see for example, Baxendale 1952). Formation of the free radical is dictated by the difficulty of removing more than one electron from the catalyst molecule. We have suggested that the usual radical chain mechanism is not the principal course of reaction in the decomposition of $H_2O_2$ by certain 6-coordinate $Ru^{II}$ complexes which can lose two electrons to $H_2O_2$ (Dwyer, King, and Winfield 1959). We wish to show that an ionic mechanism is thermodynamically feasible, and is indeed significantly favoured if a catalyst is chosen which has appropriate redox characteristics.

## II. Oxidation of Catalyst

For the oxidation of $Ru^{II}$ by $H_2O_2$ we have the alternatives (**1**) and (**2**):†

$$Ru^{II}+H_2O_2 \rightarrow Ru^{IV}+\bar{O}H+\bar{O}H, \quad \ldots\ldots\ldots\ldots \quad (1)$$

$$Ru^{II}+H_2O_2 \rightarrow Ru^{III}+\dot{O}H+\bar{O}H, \quad \ldots\ldots\ldots\ldots \quad (2)$$

where $Ru^{II}$ represents a ruthenium complex of the kind described by Dwyer, King, and Winfield (1959). Reaction (**2**) is the well-known Haber and Weiss first step in $H_2O_2$ decomposition, for example as catalysed by ferrous ion (Weiss 1952).

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† It is unnecessary to consider here the reaction

$$Ru^{III}+H_2O_2 \rightarrow Ru^{IV}+\bar{O}H+\dot{O}H,$$

since it will have a less favourable free-energy change than (**2**). Likewise in Section II, we shall not have to consider the reduction of $Ru^{III}$ by $H_2O_2$.

*Reaction* (**1**).—A catalyst is chosen for which

$$Ru^{II} \rightarrow Ru^{IV} + 2e \qquad E^0 = -0{\cdot}8 \text{ V } (\Delta G^0 = 36{\cdot}9 \text{ kcal}). \quad \ldots . \quad (3)$$

In order to obtain a cycle, a potential in the vicinity of $-0{\cdot}8$ V is necessary, so that the free-energy change for oxidation of $Ru^{II}$ to $Ru^{IV}$ by $H_2O_2$ is comparable in magnitude to that for the reduction of $Ru^{IV}$ to $Ru^{II}$ by $H_2O_2$.

By summing reaction (**3**) with the following reactions from Latimer (1952):

$$H_2O_2 \rightarrow \dot{O}H + \dot{O}H \qquad \Delta G^0 = 48{\cdot}7 \text{ kcal},$$
$$2(\dot{O}H + e \rightarrow 2\bar{O}H \qquad \Delta G^0 = -45{\cdot}5 \text{ kcal}),$$

we obtain for (**1**)

$$\Delta G^0 = -5{\cdot}4 \text{ kcal}.$$

To obtain $\Delta G$ we make use of the following steady-state concentrations, which are experimental values except for those marked with an asterisk. There is no means for readily determining these; we have chosen limiting values which tend to make the calculation of $\Delta G$ favourable to reaction (**2**) rather than to the alternative mechanism which we are suggesting:

| | | |
|---|---|---|
| $H_2O_2$ $10^{-2}$M; | $\bar{O}H$ $10^{-7}$M; | $Ru^{II}$ $10^{-4}$M; |
| $O_2$ 0·2 atm; | | $Ru^{III}$ $10^{-5}$M;* |
| $Ru^{IV}$ $10^{-4}$M; | $\dot{O}OH$ $10^{-10}$M;* | $\dot{O}H$ $10^{-10}$M;* |

Temperature 30 °C

Correcting for concentration we obtain

$$\Delta G = \Delta G^0 - 16{\cdot}7 \text{ kcal}$$
$$= -22{\cdot}1 \text{ kcal}.$$

*Reaction* (**2**).—For removal of the first electron from the $Ru^{II}$ complex of reaction (**3**), $E^0$ is likely to be in the range $-0{\cdot}2$ to $-0{\cdot}8$ V. Reaction (**2**) is favoured by a small value for $-E^0$; we shall therefore assume:

$$Ru^{II} \rightarrow Ru^{III} + e \qquad E^0 = -0{\cdot}2 \text{ V } (\Delta G^0 = 4{\cdot}6 \text{ kcal}).$$

Making use of the following free energies of reaction (Latimer 1952):

$$H_2O_2 \rightarrow \dot{O}H + \dot{O}H \qquad \Delta G^0 = 48{\cdot}7 \text{ kcal},$$
$$\dot{O}H + H^+ + e \rightarrow H_2O \qquad \Delta G^0 = -64{\cdot}6 \text{ kcal},$$
$$H_2O \rightarrow H^+ + \bar{O}H \qquad \Delta G^0 = 19{\cdot}1 \text{ kcal},$$

we then obtain for reaction (**2**)

$$\Delta G^0 = 7{\cdot}8 \text{ kcal},$$

and

$$\Delta G = -14{\cdot}5 \text{ kcal}.$$

It will be clear from the estimated free-energy changes that even for a catalyst which requires only 0·2 V for removal of one electron the equilibria

favour more decomposition of $H_2O_2$ via reaction (1) than by (2). In the special case in which the first electron is almost as difficult to remove as the second, production of hydroxyl radicals could well be negligible. It is tempting to speculate that this might prove to be the situation with catalase.

### III. Reduction of Catalyst

Here we have the two alternatives:

$$Ru^{IV}+H_2O_2 \rightarrow Ru^{II}+O_2+2H^+, \quad \ldots\ldots\ldots\ldots \quad (4)$$

$$Ru^{IV}+H_2O_2 \rightarrow Ru^{III}+\dot{O}OH+H^+. \quad \ldots\ldots\ldots\ldots \quad (5)$$

*Reaction* (4).—From reaction (3) and the following taken from Latimer (1952)

$$H_2O_2 \rightarrow O_2+2H^++2e \qquad \Delta G^0=31\cdot5 \text{ kcal},$$

we find $\Delta G^0=-5\cdot4$ kcal. Making corrections for the concentrations of reactants and products,

$$\Delta G=\Delta G^0-17\cdot8=-23\cdot2 \text{ kcal}.$$

*Reaction* (5).—Making use of

$$H_2O_2 \rightarrow \dot{O}OH+H^++e \qquad \Delta G^0=34\cdot6 \text{ kcal},$$

(Latimer 1952) and

$$Ru^{IV}+e \rightarrow Ru^{III} \qquad \Delta G^0=-32\cdot3 \text{ kcal}$$

(in accordance with the previous assumptions for the potentials) we obtain

$$\Delta G^0=2\cdot3 \text{ kcal},$$

and

$$\Delta G=-20 \text{ kcal}.$$

Thus for the reduction as well as for the oxidation, the 2-electron mechanism is favoured by the thermodynamics.

### IV. Discussion

Dwyer, King, and Winfield (1959) found that the 1-electron oxidation of $Ru^{II}$ yielded a blue $Ru^{III}$ complex ($Ru_b^{III}$) while 1-electron reduction of $Ru^{IV}$ yielded green $Ru_g^{III}$. The nature of the ligand alteration which was responsible for the green form has not been determined, but the important points were established: (i) the oxidation of $Ru^{II}$ via $Ru^{III}$ to $Ru^{IV}$ is not strictly reversible; (ii) the electrolytic reduction of $Ru_g^{III}$ is much slower than its oxidation.

Both $Ru^{III}$ complexes were formed to some extent during the catalysed decomposition of $H_2O_2$, but whether by interaction of $Ru^{II}$ and $Ru^{IV}$, or by direct action of $H_2O_2$, could not be determined. We have therefore to consider the possibility that the mechanism (1), (4), which appears to be in harmony with the experimental and thermodynamical results, was accompanied by a significant amount of $H_2O_2$ decomposition via free radicals (i.e. via the more commonly accepted mechanism).

By analogy to the radical chain mechanisms described by Baxendale (1952), Weiss (1952), and others we can write for the ruthenium catalyst the reactions (6-11) in addition to (2) and (5) given already:

$$Ru^{II} + \dot{O}H \rightarrow Ru_b^{III} + \bar{O}H, \quad \ldots\ldots\ldots\ldots (6)$$

$$Ru_b^{III} \text{ or } Ru_g^{III} + \dot{O}H \rightarrow Ru^{IV} + \bar{O}H, \quad \ldots\ldots\ldots\ldots (7)$$

$$H_2O_2 + \dot{O}H \rightarrow \dot{O}OH + H_2O, \quad \ldots\ldots\ldots\ldots (8)$$

$$Ru_b^{III} + H_2O_2 \rightarrow Ru^{IV} + \bar{O}H + \dot{O}H, \quad \ldots\ldots (9)$$

$$Ru^{IV} + \dot{O}OH \rightarrow Ru_g^{III} + O_2 + H^+, \quad \ldots\ldots (10)$$

$$Ru_b^{III} + \dot{O}OH \rightarrow Ru^{II} + O_2 + H^+. \quad \ldots\ldots\ldots (11)$$

Since the reduction of $Ru_g^{III}$ is very sluggish, we may deduce that the free radical mechanism, unaccompanied by the ionic cycle (1), (4) would quickly deplete the solution of $Ru^{II}$ so that the catalysis would then depend upon the reaction

$$Ru_g^{III} \rightleftarrows Ru^{IV} + e. \quad \ldots\ldots\ldots\ldots\ldots (12)$$

It would therefore proceed slowly at best since the redox potential of (12) is not favourable to the reaction

$$Ru_g^{III} + H_2O_2 \rightarrow Ru^{IV} + \bar{O}H + \dot{O}H.$$

This conclusion is in accord with the experimental observation that under steady-state conditions most of the ruthenium can be in the $Ru^{II}$ state. But there is no apparent reason why some free radical reactions will not proceed as long as $Ru^{IV}$ is being reduced to $Ru^{II}$ by the ionic reaction (4). The most important are expected to be (2), (5), (6), (7), (8), and (10).

It is seen in Sections II and III that the extent to which the catalysis is carried by a free radical mechanism can be expected to depend upon the redox potentials of the catalyst and the speed or completeness with which OH and OOH radicals produced in (2) and (5) can be removed by reactions (6), (7), and (10). The latter are expected to be very fast and to have large values of $-\Delta G$, thus depressing the free radical concentration to a low level; hence the low steady-state value of $10^{-10}$M used in Sections II and III.

To illustrate how the redox characteristics of the catalyst can influence the course of $H_2O_2$ decomposition, let us consider a hypothetical metal complex M, which has the following characteristics as a result of interaction of metal and ligands:

$$M^{II} \rightarrow M^{IV} + 2e \qquad E^0 = -0{\cdot}8 \text{ V},$$

$$M^{II} \rightarrow M^{III} + e \qquad \Delta G^0 = 18 \text{ kcal}, \quad \ldots\ldots (13)$$

$$M^{III} \rightarrow M^{IV} + e \qquad \Delta G^0 = 18{\cdot}9 \text{ kcal}, \quad \ldots\ldots (14)$$

$$M^{IV} + e \rightarrow M^{III} \qquad \Delta G^0 = -10 \text{ kcal}, \quad \ldots\ldots (15)$$

$$M^{III} + e \rightarrow M^{II} \qquad \Delta G^0 = -26{\cdot}9 \text{ kcal}. \quad \ldots\ldots (16)$$

The approximately equal values for $\Delta G^0$ in (13) and (14) (whereas usually the free energy required for removal of the first electron is considerably less than for the second) could result in suppression of OH radical production during oxidation

of $M^{II}$ by $H_2O_2$, as pointed out earlier. For the back reaction in which $M^{IV}$ is reduced by $H_2O_2$ the small value of $-\Delta G^0$ in reaction (**15**) would tend to block production of OOH radicals, and favour the direct 2-electron reduction to $M^{II}$. It is conceivable that this is the situation for the enzyme catalase (Cat.), and that when it loses two electrons to form Cat.I the latter can be reduced quickly by a 2-electron process to Cat., while a 1-electron reduction is accompanied by ligand reorganization which stabilizes an oxidation state between that of Cat.I and Cat. (see King and Winfield 1959).

In conclusion, it must be admitted that there may be kinetic barriers to single step 2-electron oxidations and reductions, and that most oxidations proceed in 1-electron steps. But under special circumstances, the chemical equilibria can be significantly in favour of the 2-electron process which will therefore predominate when suitably catalysed.

## V. Acknowledgments

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# A THEORETICAL STUDY OF THE CHEMISTRY OF FURAN, PYRROLE, BENZOFURAN, INDOLE, DIBENZOFURAN, AND CARBAZOLE

By R. D. Brown* and B. A. W. Coller*

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*Summary*

It is shown that it is possible, within the framework of the simple Hückel molecular-orbital method, to account fully for all observations on electrophilic substitution in furan, pyrrole, benzofuran, indole, dibenzofuran, and carbazole, in terms of the $\pi$-electron distributions in these compounds. The values of coulomb parameters required to obtain this correlation are physically reasonable and in particular are in agreement with values found independently by a variable electronegativity self-consistent field calculation on pyrrole.

The previously puzzling difference in orientation of substitution in benzofuran and indole is accounted for in the present study in terms of a difference in the auxiliary inductive effects of the two hetero-atoms. It seems possible to attribute this difference ultimately to differences in the CO and CN bond lengths and to differences in the effective nuclear charges for $2p\pi$ atomic orbitals on the two hetero-atoms.

Attention is drawn to an " isoprotonic principle " for estimating the value of the primary coulomb parameter of a hetero-atom. (It is probably a special case of a more general principle—that the primary coulomb parameter is predominantly determined by the charge on the core and scarcely depends upon the nature of the nucleus at the centre of the core.)

A spurious Hückel molecular-orbital model for the oxygen heterocycles is noted. It accounts for the chemistry of the heterocycles but represents a physically unsatisfactory view of the electronic properties of oxygen in these compounds.

## I. Introduction

The simple Hückel molecular-orbital treatment of aromatic hydrocarbons has been surprisingly successful in accounting for, among other things, the chemical properties of these compounds (Coulson 1951 ; Brown 1952 ; Longuet-Higgins 1957 ; Dewar 1958). The interpretation of the chemistry of heterocyclic compounds, even when confined to electrophilic substitution, has not been so completely successful, some apparently anomalous orientations being observed. It is possible to account for some of these in terms of complications in the mechanisms of substitution (Bassett and Brown 1954 ; Brown 1958 ; Brown and Harcourt 1959) but others, notably connected with furan, pyrrole, and their benzologues, remain. In the present paper it will be shown that these can be resolved by suitable choice of electronegativity parameters.

The present study also throws light on the problem of auxiliary inductive parameters. These coulomb parameters imply that carbon atoms adjacent to hetero-atoms are more electronegative towards $\pi$-electrons than are carbon atoms

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whose neighbours in the aromatic system are carbon atoms also. They were originally introduced by Whelaud and Pauling (1935) primarily to obtain calculated π-electron distributions in pyrrole and furan which accorded with the observed orientation of electrophilic substitution. It was subsequently demonstrated (Brown 1955) that the orientation could be accounted for in terms of localization energies without invoking auxiliary inductive effects (and thus the number of empirical parameters in the theory could be reduced !). However, evidence is accumulating (Bassett and Brown 1954 ; Brown and Heffernan 1956*a* ; Brown 1958 ; Brown and Harcourt 1959) that for all but very unreactive substrates the orientation of electrophilic substitution in heterocycles follows the π-electron distribution rather than atom-localization energies. The present study provides further evidence that auxiliary inductive parameters are needed to obtain a satisfactory interpretation of substitution in heterocycles.

## II. Method of Calculation

The simple Hückel molecular-orbital procedure, neglecting overlap integrals, was used, the calculations being directly comparable with our previous heterocycle calculations (Brown 1955 ; Brown and Heffernan 1956*a*, 1956*b*). The standard value β was assumed for all carbon-carbon bonds and the CX resonance integral, $\beta_{CX}$ (X representing the hetero-atom), was taken to be $1{\cdot}1\beta$ since this seems to be the appropriate value for CO bonds (Brown and Heffernan 1958) and is probably close to the optimum value for CN bonds too. However, some parallel calculations were performed for the *cyclo*pentadienyl and indenyl heterosystems using $\beta_{CX}=\beta$ to determine the sensitivity of our results to the chosen value of $\beta_{CX}$. The results, shown in Tables 1 and 2, indicate that the π-electron densities, on which the ensuing analysis is primarily based, are insensitive to the value of $\beta_{CX}$.

Table 1

π-ELECTRON DENSITIES FOR FURAN AND PYRROLE

| *h* | 2-Position | | 3-Position | |
|---|---|---|---|---|
| | *k**=1·1 | 1·0 | 1·1 | 1·0 |
| 2·5 | 1·0106 | 1·0074 | 1·1198 | 1·1062 |
| 0 | 1·2038 | 1·2000 | 1·2075 | 1·2000 |
| —3·75 | 1·6006 | 1·6154 | 1·2720 | 1·2725 |

* $k=\beta_{CX}/\beta$.

The difference between the coulomb integral $\alpha_X$ of the hetero-atom and a carbon atom not adjacent to X is conveniently expressed in terms of a parameter *h* such that $\alpha_X=\alpha+h\beta$. The coulomb integrals of those carbon atoms adjacent to X are similarly expressed as $\alpha_{C'}=\alpha+h'\beta$, non-zero values of $h'$ representing the auxiliary inductive effect of the hetero-atom upon the neighbouring atoms. The coulomb integrals of all other carbon atoms were assigned the standard value α. It has been suggested (Dewar 1949) that the auxiliary inductive effect

extends beyond the immediate neighbours of hetero-atoms, falling off in geometric progression around the conjugated system. This proposal was based on the assumption that the effect arises from polarization of the σ-bonds in the conjugated system. However, a detailed study of pyrrole and several other heterocyclic systems by the variable electronegativity self-consistent field (VESCF) method (Brown and Heffernan 1959) has shown that σ-bond polarization is negligible and that the auxiliary inductive effect arises from core attraction terms and is limited to the immediate neighbours of hetero-atoms.

TABLE 2

π-ELECTRON DENSITIES FOR BENZOFURAN AND INDOLE

| $h$ | 2-Position | | 3-Position | | 4-Position | | 5-Position | | 6-Position | | 7-Position | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $k^*=$ 1·1 | 1·0 | 1·1 | 1·0 | 1·1 | 1·0 | 1·1 | 1·0 | 1·1 | 1·0 | 1·1 | 1·0 |
| 2 | 0·9564 | 0·9584 | 1·1602 | 1·1416 | 1·0216 | 1·0196 | 1·0364 | 1·0323 | 1·0256 | 1·0224 | 1·0417 | 1·0378 |
| 0 | 1·1117 | 1·1118 | 1·2631 | 1·2516 | 1·0351 | 1·0342 | 1·0614 | 1·0591 | 1·0609 | 1·0590 | 1·0360 | 1·0343 |
| −2 | 1·4376 | 1·4609 | 1·3357 | 1·1921 | 1·0583 | 1·0619 | 1·0800 | 1·0791 | 1·1338 | 1·1438 | 0·9928 | 0·9887 |

* $k=\beta_{CX}/\beta$.

Two series of computations were performed. In the first series the auxiliary inductive effect was assumed to be negligible (i.e. $h'=0$) and π-electron densities and localization energies were computed for each compound over the whole range of $h$. In the second series calculations were performed for a range of $h'$, keeping $h$ constant at the value +2. A recent VESCF study of pyrrole (Brown and Heffernan 1959) indicates that this value is appropriate for secondary nitrogen. The "isoprotonic principle"—that the coulomb integrals for isoprotonic cores are similar, as demonstrated by the very small difference of coulomb integral of pyridinic nitrogen and isoprotonic CH in both pyridine (Brown and Heffernan 1957, 1959) and the pyrrole anion (Brown and Heffernan 1959), may be used to suggest that for oxygen, which is isoprotonic with NH, the value of $h$ will be around +2 also.

## III. RESULTS AND DISCUSSION

### (a) *Furan and Pyrrole*

Electrophilic substitution in furan cannot be achieved in strongly acidic media because the latter cause resinification. It is reasonable to assume that it is the protonated oxonium system which is involved in the resinification and that the electrophilic displacements on carbon which have been observed in less acidic media take place in the unprotonated heterocycle.* Halogenation (Henninger 1886; Cass and Copelin 1947), Friedel-Crafts reaction (Reichstein

* We therefore have to account for orientations in terms of a theoretical treatment of furan itself rather than, say, a protonated furan cation in which the protonated oxygen would have very different coulomb parameters from those of unprotonated oxygen. Similar considerations apply of course to the other heterocycles considered in this paper.

1930), nitration (Rinkes 1938), and sulphonation (Kazitsyna 1946) all occur preferentially at the 2-position. Pyrrole shows the same orientation of electrophilic substitution (Fischer and Hepp 1886 ; Mazzara and Borgo 1905*a*, 1905*b* ; Rinkes 1934), there being little doubt that the heterocycle is reacting as the uncharged molecule. However in pyrrole the preference for α- rather than β-substitution is not so severe as in furan.

The observed orientations can be accounted for in terms of electrophilic localization energies (Fig. 1), without invoking auxiliary inductive effects, for positive values of $h$, that is, assuming the hetero-atom to be more electronegative than carbon. However, all the 5-membered ring heterocycles are very reactive

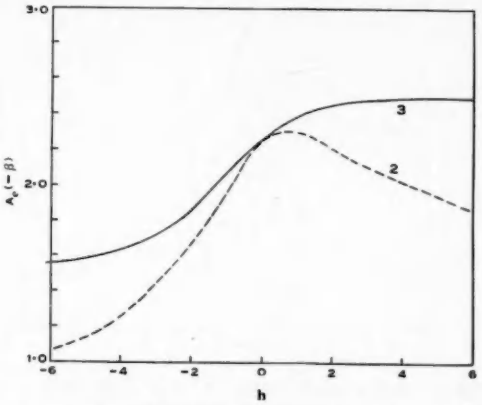



Fig. 1.—Atom-localization energies for electrophilic substitution in furan and pyrrole.

towards electrophils so that the orientations would be expected (see Section I) to follow the π-electron densities rather than localization energies. When the auxiliary inductive effect is ignored (Fig. 2) the electron densities fall in the appropriate order only for negative $h$, which corresponds to assuming that the hetero-atom is less electronegative than carbon. This is highly unlikely since in both cases the hetero-atom presents a higher core charge (+2) to the π-electrons than do the carbon atoms (+1) and for secondary nitrogen the VESCF calculations show that the hetero-atom is considerably more electronegative than the carbons.* When the auxiliary inductive parameter is considered using a

* Some time ago (Bassett, Brown, and Penfold 1956) we suggested on the basis of dipole moment data and of orientation of substitution that tertiary nitrogen and oxygen may sometimes be less electronegative than carbon. Further investigation (Brown and Heffernan 1959) has confirmed this suggestion in the case of tertiary nitrogen but it now seems incorrect for oxygen. The dipole moment of furan must be interpreted differently now that there is good evidence (Brown and Heffernan 1958, 1959) that there is virtually no σ-bond polarization in these heterocycles. Although the orientation of substitution in the oxygen heterocycles can be accounted for using a negative value of $h$, as is shown in detail in the present paper, this must now be regarded as a " spurious model " for the heterocycles.

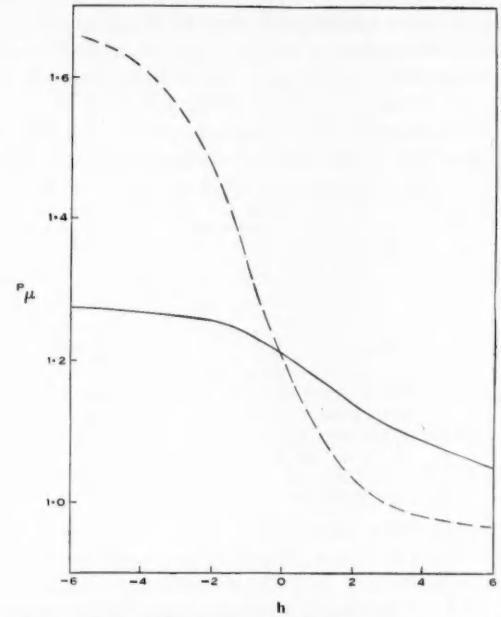



Fig. 2.—π-Electron densities for furan and pyrrole (no auxiliary inductive effect).

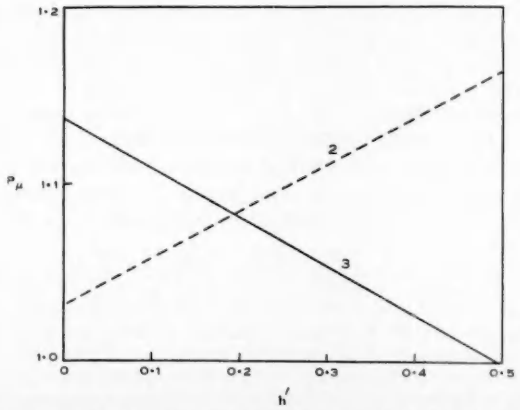



Fig. 3.—π-Electron densities in furan and pyrrole as a function of the auxiliary inductive parameter.

physically acceptable value for $h$ (Fig. 3), the electron densities fall into line with the observed orientation for $h' > 0 \cdot 19$. The details of electrophilic substitution in these two heterocycles may thus be understood if a greater auxiliary inductive effect is attributed to oxygen than to nitrogen and if for both the critical value of $h' = 0 \cdot 19$ is exceeded.

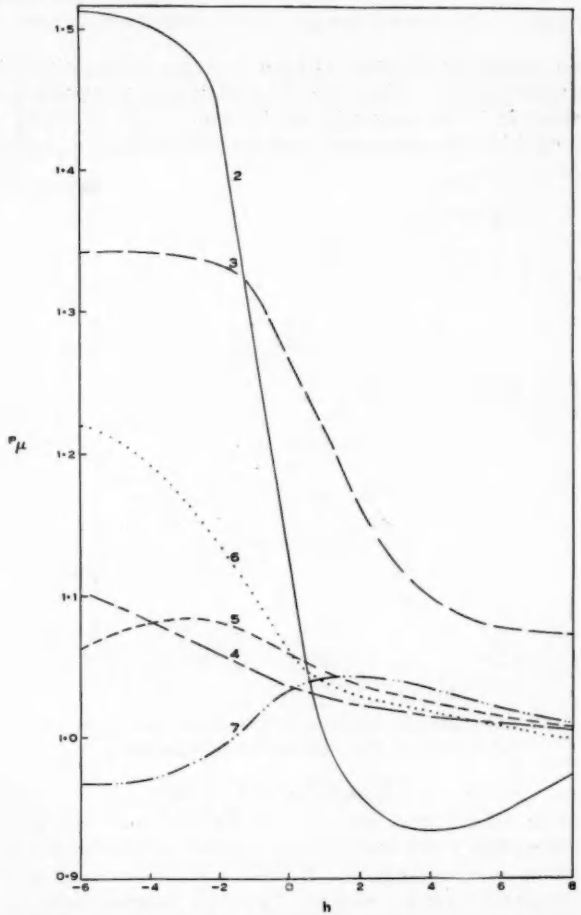
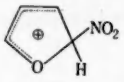



Fig. 4.—π-Electron densities in benzofuran and indole (no auxiliary inductive effect).

The nitration of furan is of interest because it has been observed (Marquis 1905) to proceed through a 2,5-addition of nitronium acetate; it has recently been shown (Michels and Hayes 1958) that the conversion of this to the nitrofuran

is a general base-catalysed process. This indicates that initial attack by the nitronium electrophil produces a relatively long-lived intermediate I which is




(I)

attacked more rapidly by nucleophils at the carbon in the 5-position than at the hydrogen on the 2-position. Since two of the original six π-electrons are localized at the 2-position in I, the oxygen is now in an environment similar to that in acrolein and addition of a nucleophil preferentially at the " carbonyl " carbon is to be anticipated.

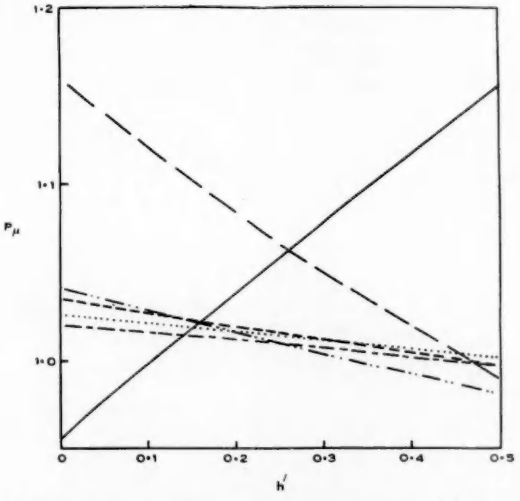



Fig. 5.—π-Electron densities of benzofuran and indole as a function of the auxiliary inductive parameter.

### (*b*) *Benzofuran and Indole*

Electrophilic substitutions such as nitration (Stoermer and Richter 1902), acetylation (Smith 1937), and sulphonation (Kazitsyna 1953) occur preferentially at the 2-position of benzofuran, the 3-position being the next most reactive (Adams and Rindfusz 1919) and then the 6-position (Gilman, Smith, and Cheney 1935). In contrast, electrophilic substitution in indole occurs preferentially at the 3-position (Sumpter and Miller 1954), the 2- and 5-positions being next in order of reactivity (Plant and Tomlinson 1933). The conditions of reaction in these cases point to substitution in the uncharged heterocycles and this has been explicitly demonstrated in the case of diazonium coupling in indole (Binks and Ridd 1957).

The π-electron densities for these heterocycles, calculated with no auxiliary inductive effect, are shown in Figure 4. They fall in the order $2>3>6$ for $h<-1\cdot3$ and in the order $3>2>5$ only for $0<h<0\cdot5$. Thus we can account for the observed orientations if secondary nitrogen is assumed to be slightly more electronegative than carbon (i.e. of similar electronegativity to a pyridinic nitrogen) and if oxygen is assumed to be considerably less electronegative than carbon, but both of these assumptions and particularly the second, seem very unlikely.

If the hetero-atoms are taken to be appreciably more electronegative than carbon ($h=+2$) and the auxiliary inductive effect is considered (Fig. 5) then the

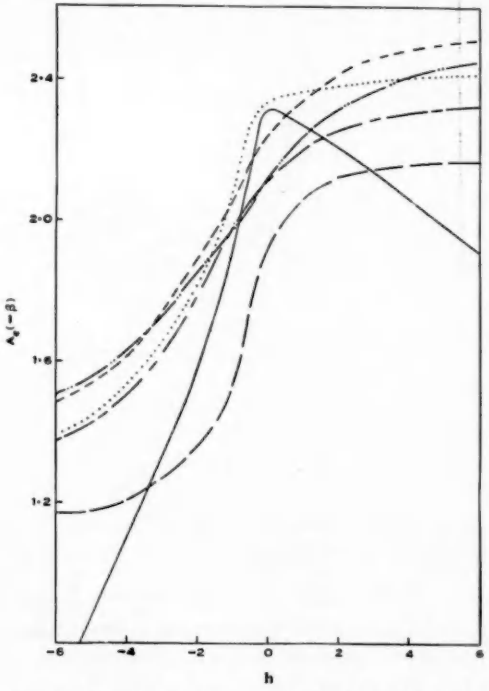



Fig. 6.—Atom-localization energies for electrophilic substitution in benzofuran and indole.

order of electron densities required for benzofuran is obtained for $0\cdot32<h'<0\cdot46$ and that required for indole for $0\cdot16<h'<0\cdot26$.

For comparison the electrophilic localization energies are shown in Figure 6. The ease of localization is $2>3$ for $h>2\cdot9$ or $<-3\cdot4$, but the 6-position is not next in ease of localization and for no value of $h$ does the order of ease of localizing atoms become $3>2>5$.

The sulphonation of indole by pyridine–sulphur trioxide gives indole 2-sulphonic acid (Terent'ev and Golubeva 1946). However, the reaction appears to involve initial formation of the *N*-sulphonic acid, so that the orientation of

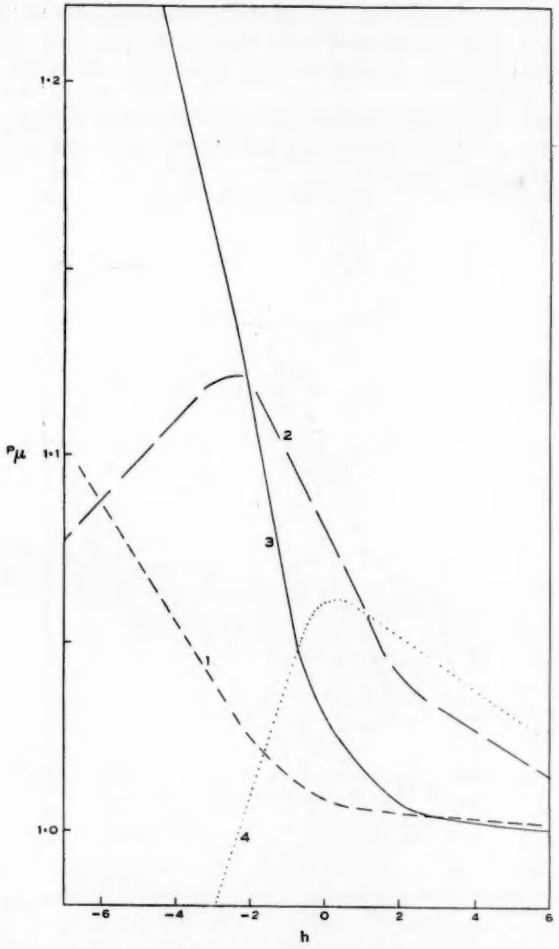



Fig. 7.—π-Electron densities in dibenzofuran and carbazole (no auxiliary inductive effect).

the final product may be limited sterically to a 1,2-rearrangement. Alternatively the final isomerization might be regarded as an intramolecular electrophilic substitution in the indole anion. In either case the deviation from the normal orientation of electrophilic substitution is understandable.

### (c) *Dibenzofuran and Carbazole*

Monosubstitution by electrophils in dibenzofuran (the numbering system for dibenzofuran and carbazole is shown in II) has been reported to occur at the 2-position (Gilman, Smith, and Oatfield 1934; Gilman and Young 1935). However, a very careful study of the orientation of nitration in acetic anhydride by Dewar and Urch (1957) has shown that the order of reactivity of the positions is 2=3>1>4. For carbazole the order 2>4>3, 1 has been reported for several

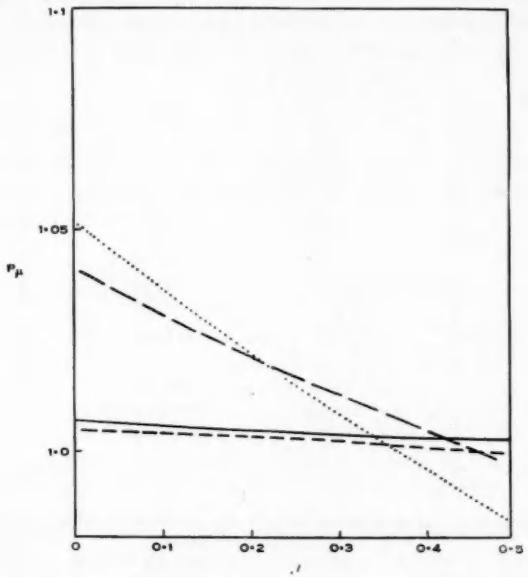
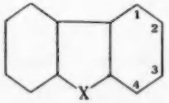



Fig. 8.—π-Electron densities in dibenzofuran and carbazole as a function of the auxiliary inductive parameter.

electrophilic substitutions (Lindemann 1924; Tucker 1926; Dunlop and Tucker 1939; Meitzner 1935; Plant, Rogers, and Williams 1935). However a recent detailed study of the nitration of carbazole in acetic anhydride (Dewar and Urch




(II)

1958) has shown that the order is 2>4>3>1. The mildly acidic conditions employed for the nitrations make it very probable that the uncharged molecular species are involved in the case of both dibenzofuran and carbazole.

The appropriate order of $\pi$-electron densities (Fig. 7) for dibenzofuran is produced, when the auxiliary inductive effect is neglected, for $h=-2\cdot1$, and for carbazole for $-0\cdot7<h<+1\cdot1$, the latter being perhaps acceptable but the

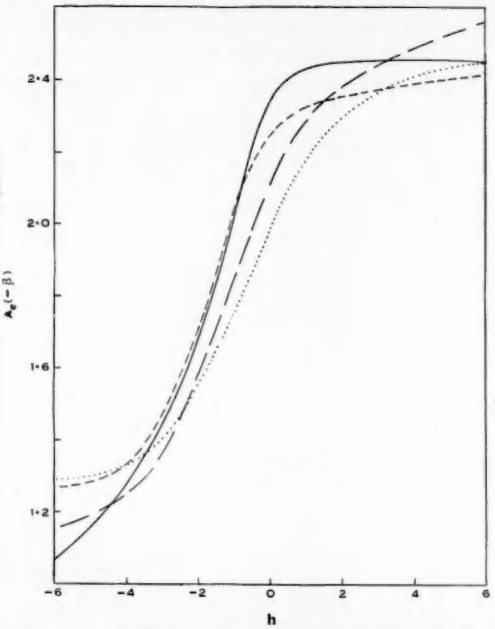



Fig. 9.—Atom-localization energies for electrophilic substitution in dibenzofuran and carbazole.

value for oxygen being again unreasonable. However, the orientations can be satisfactorily accounted for (Fig. 8) with $h=2$ and, for dibenzofuran, $h'\approx0\cdot42$, while for carbazole, $0\cdot21<h'<0\cdot34$. Furthermore the relative levels of reactivity of carbazole and dibenzofuran positions (Table 3) may be accounted for in terms

TABLE 3

PARTIAL RATE FACTORS FOR NITRATION IN ACETIC ANHYDRIDE

| Benzofuran | | Carbazole | |
|---|---|---|---|
| Position | Rate* | Position | Rate* |
| 1 | 47 | 1 | Small |
| 2 | 94 | 2 | $7\cdot8\times10^4$ |
| 3 | 94 | 3 | $0\cdot11\times10^4$ |
| 4 | Small | 4 | $3\cdot2\times10^4$ |

* Relative to a benzene position at 0 °C.

of comparative π-electron densities, the electron densities of the more reactive carbazole being higher than for dibenzofuran.

The electrophilic localization energies (Fig. 9) cannot be correlated with the observed orientations for any value of $h$ but again there seems little doubt that these compounds are sufficiently reactive for the orientations to follow charges rather than localization energies.

## IV. Conclusion

The analysis of electrophilic substitution in these five-membered ring heterocycles leads to several conclusions. Firstly, one can account satisfactorily for the observations on all compounds by using the coulomb parameters:

| | | |
|---|---|---|
| O | $h=2$ | $h'=0\cdot42$ |
| NH | $h=2$ | $h'=0\cdot25$ |

the data on which these values are based being summarized in Table 4. In the case of secondary nitrogen the value for $h'$ is in excellent agreement with that suggested by a VESCF treatment of pyrrole.

Table 4

Auxiliary Inductive Coulomb Parameters

| Heterocycle | Parameter Range* | Heterocycle | Parameter Range* |
|---|---|---|---|
| Pyrrole .. .. | 0·2 –0·25, say | Furan .. .. | >0·25, say |
| Indole .. .. | 0·16–0·26 | Benzofuran .. | 0·32–0·46 |
| Carbazole.. .. | 0·21–0·34 | Dibenzofuran .. | ≈0·42 |

* Based on the value $h=+2$ for the primary coulomb parameter.

It is possible to account for the greater auxiliary inductive effect of oxygen than secondary nitrogen as follows. The factor primarily responsible for the auxiliary inductive effect has been identified, through VESCF investigations of heterocyclic systems, as the core attraction terms in the diagonal elements of the VESCF Hamiltonian matrix. The difference in coulomb integrals of carbons adjacent to O and to NH respectively arises mainly from differences in the term $(\mu \mid V_X \mid \mu)$, representing the electrostatic attraction of the hetero-atom core for an electron occupying the $2p\pi$ orbital on the adjacent carbon atom, $\mu$. This term varies rapidly with the internuclear distance between X and $\mu$, with the core charge on X and with the effective nuclear charge, $Z_X$, of the $2p\pi$ orbital on X. The core charge is +2 for both O and NH. It has been found (Bak 1955) that the CO length in furan is slightly shorter than the CN length in pyrrole, and $Z_X$ is greater for oxygen than for nitrogen, both of these factors tending to make the auxiliary inductive effect of oxygen greater than that of secondary nitrogen. Thus the conclusions derived subjectively in the present study of five-membered ring heterocycles appear to be physically reasonable. It is hoped to obtain more detailed information from a VESCF treatment of furan which is now being undertaken.

In the case of oxygen there is also a "spurious Hückel model" which accounts for electrophilic substitution in the three heterocycles, namely, $h=-2\cdot1$, $h'=0$. This model was partly responsible for the suggestion of an electronegativity reversal in these oxygen heterocycles (Bassett, Brown, and Penfold 1956) which must now be rejected as being physically unlikely on the grounds of the isoprotonic principle and the VESCF results for pyrrole.

## V. Acknowledgments

Most of the computations were performed on the University of Melbourne computer CSIRAC. We are grateful to Mr. A. Jones of the Department of Mathematics for providing the eigenvalue-eigenvector programme employed, and to the staff of the computer laboratory for facilitating the calculations. One of us (B.A.W.C.) gratefully acknowledges the award of a Dunlop Research Scholarship.

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# THE SURFACE PROPERTIES OF ALCOHOLS CONTAINING STERICALLY HINDERED HYDROXYL GROUPS

By G. E. Hibberd* and A. E. Alexander*

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*Summary*

The surface properties of several alcohols containing sterically hindered hydroxyl groups have been studied.

Tri*cyclo*hexyl carbinol formed monolayers having a surface moment considerably greater than that of long-chain alcohols. It is suggested that this is due to the virtual inaccessibility of the C—O bond to a water molecule.

The collapse phenomenon of tri*cyclo*hexyl carbinol is quite different from that expected for a compound of m.p. 91–92 °C. It appears that the molecules collapse to form a lens or glass, rather than a crystal.

1,1,3,3-Tetraphenyl propanediol-1,3 gave monolayers of similar surface properties. The other compounds tested did not give films of measurable stability.

## I. Introduction

In the study of the surface behaviour of paraffin-chain substances, whether adsorbed from aqueous solution or spread in the form of an insoluble monolayer on water, it has been tacitly assumed that the polar group(s) responsible for the stability of the film are freely accessible to the water molecules. This assumption is supported by data on the surface moments of the paraffin-chain alcohols and acids, which change little on going from the expanded to the condensed state (e.g. Alexander 1941, 1942).

Recent studies of dielectric properties and infra-red absorption spectra have shown that certain classes of alcohols in the solid state or in organic solvents, possess largely non-associated hydroxyl groups (Meakins 1953, 1956 ; Davies and Meakins 1957 ; Cook, Davies, and Reece unpublished data). This suggested that their surface properties might well differ appreciably from those of the alcohols previously studied ; the results presented below show that this is indeed the case. In view of this interesting conclusion it is hoped to extend the study to other types of polar groups.

## II. Materials

The structural formulae of the compounds used are shown in Figure 1 ; references to their preparation and purification have been given by Meakins (1953, 1956) and by Cook, Davies, and Reece (1959).

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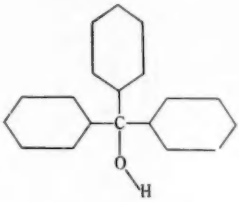
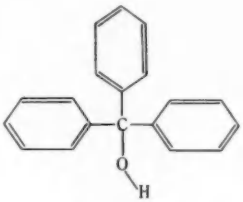
## III. Experimental Methods

For insoluble monolayer studies, a simple type of film balance with a hanging mica plate was used for measurement of surface pressures and a polonium 210 ionizing air electrode with valve electrometer for measurement of surface potentials. The spreading solvent was benzene containing *c.* 1 per cent. *n*-propanol and the substrate 0·001N hydrochloric acid.

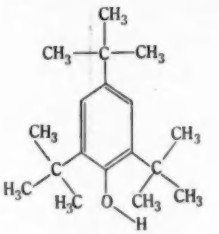
Surface moments ($\mu$) were calculated from the equation $\Delta V = 4\pi\mu/A$, where $\Delta V$ denotes surface potential and $A$ the area per molecule. Values are given in millidebye units (mD).



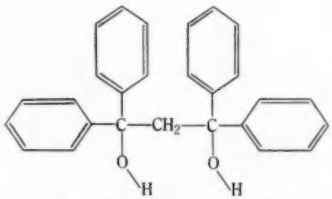
Tricyclohexyl Carbinol



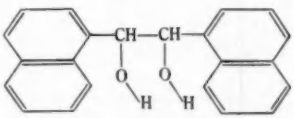
Triphenyl Carbinol



2,4,6-Tri-*tert.*-butyl Phenol



1,1,3,3-Tetraphenyl Propanediol-1,3



Dihydro-α-naphthoin

Fig. 1.—Structural formulae of the compounds studied.

Interfacial tensions between water and the benzene solutions of the various alcohols were obtained from drop-volume measurements, using an Agla micrometer syringe fitted with a stainless steel tip.

Models of the compounds studied were made up from Leybold atomic models, designed by Stuart and Briegleb.

## IV. Results and Discussion

### (*a*) *Insoluble Monolayers at the Air/Water Interface*

Initial experiments indicated that, of the five compounds examined (tricyclohexyl carbinol, 1,1,3,3-tetraphenyl propanediol-1,3, triphenyl carbinol, 2,4,6-tri-*tert.*-butyl phenol, and dihydro-α-naphthoin), only the first two appeared to give monolayers of any measurable stability. The marked difference in this respect between the very similar molecules, tricyclohexyl carbinol and triphenyl carbinol, was particularly surprising and arises, we believe, from the greater

van der Waals attraction between the aromatic rings in the latter compound (cf. m.p.'s of triphenyl carbinol 162 °C, and tri*cyclo*hexyl carbinol 91–92 °C).

An attempt was made to improve the stability of triphenyl carbinol by the technique of mixed films, but spreading with cholesterol (1 : 1) or with cetyl alcohol (1 : 3) gave no detectable improvement.

(i) *Tri*cyclo*hexyl Carbinol.*—The $\Pi/A$, $\Delta V/A$, and $\mu/A$ curves for this compound at 20 °C are shown in Figure 2, and the effect of temperature on the $\Pi/A$ curve in Figure 3.

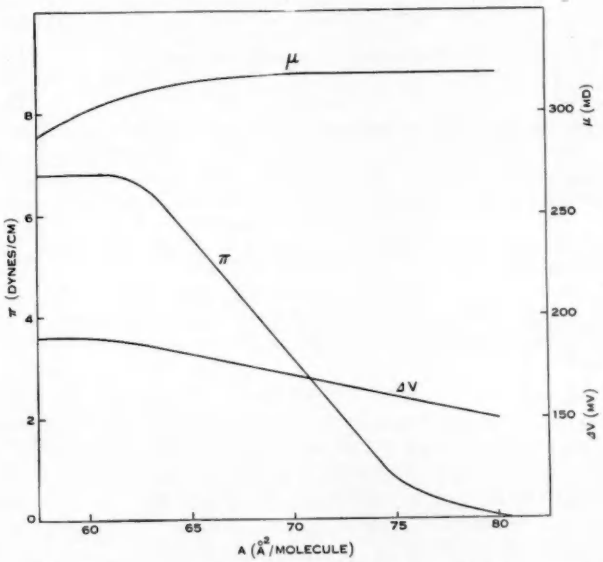



Fig. 2.—Surface pressure ($\Pi$), surface potential ($\Delta V$), and surface moment ($\mu$) as a function of area per molecule ($A$) for tri*cyclo*hexyl carbinol on 0·001N HCl ($T$, 20 °C).

The clear indication of an expanded type of film at 34·5 °C suggests that the films at lower temperatures are of the condensed type. This is supported by the agreement between the minimum area calculated from models, namely, 71 $A^2$, and the extrapolated area observed, namely 76–77 $A^2$.

Examination of Figures 2 and 3 reveals two very striking phenomena :

(i) the surface moment, $\mu$, is very substantially greater than for long-chain alcohols (319 as compared with *c.* 230 mD),*

(ii) the collapse phenomenon is quite different from the behaviour anticipated for a compound of m.p. 91–92 °C.

* Observed values depend somewhat on the area, ranging between about 220 and 250 mD (Alexander 1941 ; Schick 1957).

Figures 4 (*a*) and 4 (*b*) show the likely configuration taken up by the polar group in a long-chain normal alcohol and in tri*cyclo*hexyl carbinol in the condensed state. Taking reasonable values of 170 and 430 mD for the component dipoles

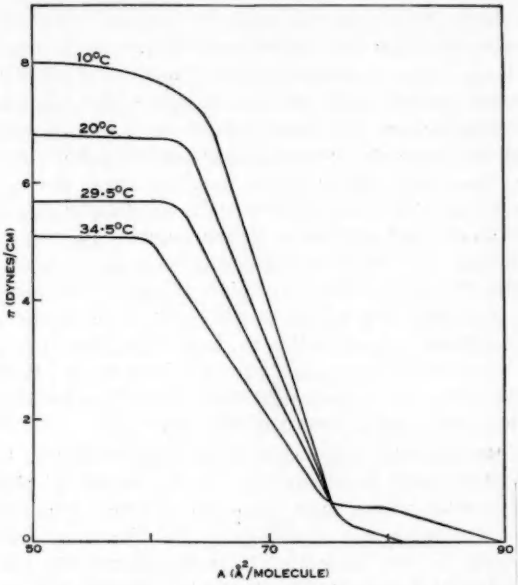



Fig. 3.—Surface pressure (Π) as a function of area per molecule (*A*) for tri*cyclo*hexyl carbinol on 0·001N HCl at various temperatures.

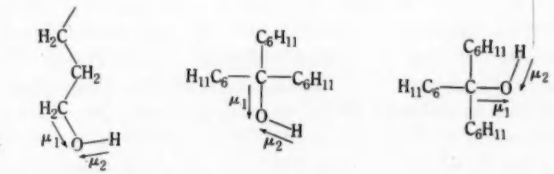



Fig. 4.—Orientation of the polar groups in a paraffin-chain alcohol and in tri*cyclo*hexyl carbinol.

$\mu_1$ and $\mu_2$ (based upon other studies of dipole moments in surface films (Alexander 1941, 1942 ; Schick 1957) and in bulk (Le Fèvre 1953)), we calculate a value of *c.* 23 mD for tri*cyclo*hexyl carbinol, that is, less than one-tenth of the observed figure. Any plausible changes in the above assumptions will not substantially

alter the final calculated moment, and it is most improbable that the bulk moments of the C—O and O—H bonds are appreciably altered by the presence of the saturated *cyclo*hexyl rings (Le Fèvre 1958, personal communication). We are therefore forced to the conclusion that the contribution to the surface moment from the C—O bond in the sterically hindered alcohol is substantially *greater* than in simple unhindered aliphatic alcohols.

Now it is known that the observed surface moment is about 15–25 per cent. of the bulk value for the same polar group (provided orientation effects are allowed for, as in the esters (Alexander and Schulman 1937)), and this is reasonably attributed to " hydration " of the constituent dipoles. Any reduction in accessibility to the water dipoles would therefore affect the surface moment, and the above results with the carbinol could be ascribed to this effect upon the C—O bond, the O—H bond still having its usual surface moment. The observed results would require an increase in the surface moment of the C—O bond from 170 to *c.* 470 mD, that is, to about two-thirds of its value in non-polar solvents (700 mD). Examination of a model of the carbinol shows the virtual impossibility of a water molecule penetrating to the carbon atom of the C—O dipole, whereas the H atom and to some extent the O atom of the O—H dipole would be freely accessible. The above interpretation of the high surface moment would not therefore appear to be an unreasonable one.

The possibility that the observed moment could arise from a configuration approximately that shown in Figure 4 (*c*) can be ruled out since this would involve the immersion of at least nine $CH_2$ groups, requiring more than 1800 cal/g-mole (Alexander and Schulman 1937). Also, the observation that the surface moment of the normal alcohols is substantially the same in the expanded and condensed states (Alexander 1941 ; Schick 1957) shows that the —O—H group has little inherent tendency to take up the configuration of Figure 4 (*c*).

Turning now to the collapse phenomenon, the constant and reproducible pressures observed would be consistent with the behaviour of a liquid or low melting solid, but the carbinol melted at 91–92 °C and crystals did not spread spontaneously on water even at a temperature of 60 °C. The collapse pressure is seen from Figure 3 to decrease with increasing temperature, which is consistent with the observations of Cary and Rideal (1925) on the spreading of long-chain compounds in the liquid state. The film behaviour can reasonably be ascribed to the carbinol molecules collapsing to form a lens rather than a crystal ; this is supported by the observation that on cooling the molten carbinol gives a glassy bead which does not crystallize readily.

(ii) *1,1,3,3-Tetraphenyl Propanediol-1,3.*—This compound also gave liquid condensed monolayers which were not very stable, although reversible below the collapse pressure (Fig. 5). The films collapsed in a very similar manner to the carbinol ; the variation of collapse pressure with temperature was also similar, although the results were not as reproducible. The collapse phenomena seem likely to arise from the same cause as with the carbinol, namely, separation of the monolayer as a lens.

The areas extrapolated from the linear part of the II-$A$ curves varied somewhat with temperature, as shown below:

| | | | | | | |
|---|---|---|---|---|---|---|
| $T$ (°C) .. .. .. | 3·0 | 6·5 | 11·2 | 15·3 | 20·2 |
| Area ($A^2$/molecule) .. .. | 83·2 | 85·5 | 87·6 | 90·3 | 92·5 |

From models the minimum calculated area is *c.* 80 $A^2$/molecule, in good agreement with the value observed at the lower temperatures.

From the surface potential measurements (Fig. 5) the surface moment at the cohering point is seen to be *c.* 640 mD. It may be coincidence that this value is exactly twice that for the carbinol with its single hydroxyl group. A

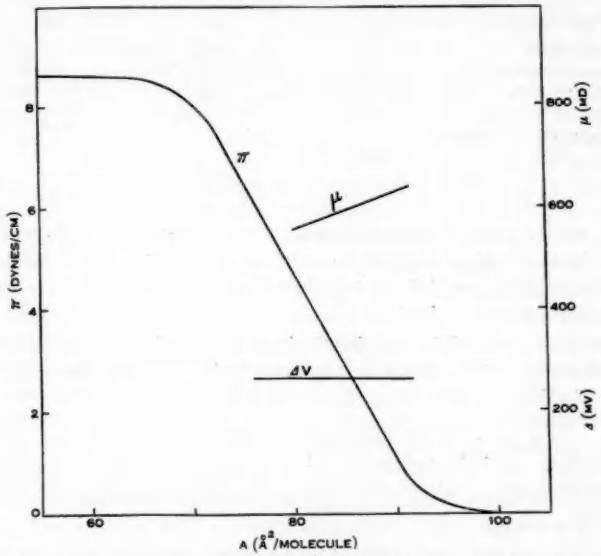



Fig. 5.—Surface pressure (Π), surface potential ($\Delta V$), and surface moment ($\mu$) as a function of area per molecule ($A$) for 1,1,3,3-tetraphenyl propanediol-1,3 on 0·001N HCl ($T$, 20 °C).

study of molecular models shows that both C—O bonds would be virtually inaccessible to the water molecules, so that it seems reasonable to interpret the high surface moment in the same manner as that advanced above for the carbinol.

### (*b*) *Adsorbed Films at the Benzene/Water Interface*

These measurements were undertaken to provide further information on the "accessibility" of the polar groups to water, for any adsorption at the interface would be reflected as a lowering in the interfacial tension. Tri*cyclo*hexyl carbinol and triphenyl carbinol were selected as most suitable on the basis of relative simplicity of structure and availability of closely related compounds. They were dissolved in benzene at a concentration of 0·056M and the interfacial

tension against water compared with the related compounds diphenyl carbinol, phenyl carbinol, di*cyclo*hexyl carbinol, and *cyclo*hexanol, as well as with cholesterol, all at the same molar concentration. (A more comprehensive study was precluded on account of the small amounts of certain compounds available.)

It is clear from the results given in Table 1, particularly from the series of the three phenyl carbinols, that surface activity is small or absent with the sterically hindered compounds, and increases as the polar group is made more accessible to the water molecules. With triphenyl carbinol no lowering in

TABLE 1

INTERFACIAL TENSION MEASUREMENTS (DYNES/CM) OF BENZENE SOLUTIONS OF ALCOHOLS (0·056M)

| | | | |
|---|---|---|---|
| Benzene/water .. .. | 35·0 | Tri*cyclo*hexyl carbinol | 32·4 |
| Triphenyl carbinol .. | 35·0 | Di*cyclo*hexyl carbinol | 29·9 |
| Diphenyl carbinol (benzohydrol) .. .. | 32·7 | *cyclo*Hexanol .. .. | 28·1 |
| Monophenyl carbinol (benzyl alcohol) .. | 29·0 | Cholesterol .. .. | 15·4 |

interfacial tension could be detected even at a concentration of 0·2M. Tri*cyclo*hexyl carbinol did give a small reduction, but again much less than the di*cyclo*hexyl carbinol, and this in turn less than *cyclo*hexanol. (The mono*cyclo*hexyl carbinol was unobtainable.)

Whilst the above data are insufficient to permit any detailed analysis, they do, however, further strengthen the conclusions reached from the insoluble monolayer studies.

## V. ACKNOWLEDGMENTS

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# THE SYNTHESIS OF THE STEREOISOMERIC HEXADECA-2,4-DIENOIC ACIDS AND THEIR *ISO*BUTYLAMIDES

By P. C. Wailes*

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*Summary*

The four stereoisomeric hexadeca-2,4-dienoic acids have been synthesized together with their *iso*butylamides and ethyl esters. The four acids, hexadeca-2,4-diynoic, hexadeca-*trans*-2-en-4-ynoic, hexadeca-*trans*-4-en-2-ynoic, and hexadeca-*cis*-4-en-2-ynoic acids were obtained as intermediates. The insecticidal activities of the *iso*butylamides of these acids have been determined.

## I. Introduction

The *iso*butylamides of certain polyunsaturated aliphatic acids have been shown to be the active constituents of a number of plant extracts possessing strong insecticidal properties which sometimes surpass those of the pyrethrins (Crombie 1955*a*, 1955*b*). The nature and location of the unsaturated groups of some of these amides have been established, but the exact structural requirements for the highest toxicity in this class of compound are still unknown. From synthetic work, largely by Crombie, it appears that derivation from a 2,4-dienoic acid is desirable but that additional structural features are likely to be required. Previous to this work only two examples of a group of four possible stereoisomeric 2,4-dienoic acids had been described and the *iso*butylamides of only one of these examples had been prepared. Allan, Jones, and Whiting (1955) had synthesized the four hexa-2,4-dienoic acids (I, R = $CH_3$) and Crombie (1955*c*) had synthesized the four deca-2,4-dienoic acids (I, R = $C_5H_{11}$). Crombie had also converted his acids into the *iso*butylamides and had reported their insecticidal properties. The isomeric *trans*-2,*trans*-4 and *trans*-2,*cis*-4-amides showed activity against the housefly (*Musca domestica*) and against the meal worm (*Tenebrio molitor*).

$$R{-}CH{=}CH{\cdot}CH{=}CH{\cdot}CO_2H$$
(I)

The *iso*butylamide of the *trans-trans*-decadienoic acid, which is the most active of the isomers, is a principal component of pellitorine, a crystalline extract of pellitory root. Nevertheless Crombie (1955*b*) was able to show that the root contained unidentified materials of even higher toxicity. He also showed that amides of the $C_{12}$ and $C_{14}$ families were present in pellitorine to a smaller extent and that the $C_{12}$ amides were inactive. Others of these naturally occurring active *iso*butylamides are known to derive from unsaturated aliphatic acids of higher carbon families with 16 or 18 atoms. It seemed desirable to prepare and measure

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the insecticidal properties of the *iso*butylamides of the 2,4-dienoic acids of one of the higher families so as to provide a further basis of comparison. Their preparation would serve also to relate the problem to the higher fatty acids of which large supplies are available.

This work describes the preparation of the four possible stereoisomeric hexadeca-2,4-dienoic acids and their *iso*butylamides.

The synthetic problem is one of choosing the best stereospecific reactions. For the introduction of the *cis*-olefinic linkages partial reduction of acetylenic bonds using Lindlar's catalyst has proved the most successful. It has been confirmed that the proportion of *trans*-isomer to which it gives rise is small, never exceeding 5 per cent., and furthermore such contaminant was readily removed either by distillation or crystallization. Other methods were tried. Reduction in boiling ethanol with a zinc-copper couple, which Crombie (1957) recently used successfully on non-conjugated acetylenes, was tested on hexadeca-*trans*-2-en-4-ynoic. Although it gave some of the desired *trans-cis*-stereoisomer the yield was less than with Lindlar's catalyst.

Allan, Jones, and Whiting (1955) as well as Crombie, Harper, and Smith (1957) have used elimination of the tosyl group to introduce *cis*-bonds in 2,4-dienoic conjugation. This elimination also proved less stereospecific and needed a reliable means for separating the *trans*- and *cis*-isomers. The introduction of a double bond by condensation between an aldehyde and an alkyl triphenyl phosphonium halide in the Wittig reaction (Wittig and Sköllkopf 1954) also produces both geometric isomers and can rarely be relied on to produce the *cis*-isomer in the greater proportion. Bohlmann and Mannhardt (1957) have referred to the synthesis of a *cis*-polyenyne with the Wittig reaction, but details have yet to be published. In work here this elegant reaction was used to prepare pentadec-3-en-1-yne which afterwards was carboxylated. The resulting mixture of *cis*- and *trans*-enynoic acids were separable almost quantitatively through the urea inclusion compound of the *trans*-acid and the *cis*-isomer was found to form 80–85 per cent. of the products. This ready separation not only made the hexadeca-*trans*-4-en-2-ynoic acid and hence the *cis*-2,*trans*-4-dienoic acid available, but also opened up an alternative route to the *cis-cis*-dienoic acid. Apart from this special use of the Wittig reaction *trans*-double bonds were introduced by well-established procedures.

The syntheses of the four hexadecadienoic acids are now given in outline.

## II. Discussion of Syntheses

### (*a*) *Hexadeca*-trans-*2*,trans-*4-dienoic Acid*

Undecyl bromide (from silver dodecanoate by a Simonini reaction (Hunsdiecker 1942)) was condensed with sodium acetylide in liquid ammonia and the resulting tridecyne (II) allowed to react, as its Grignard derivative with ethyl orthoformate. The resulting acetal (III) was partially reduced with Lindlar's catalyst in methyl acetate to 1,1-diethoxytetradeca-*cis*-2-ene (IV), which underwent geometric inversion on acid hydrolysis to give tetradeca-*trans*-2-enal (V) (Raphael and Sondheimer 1951). Ethyl 3-hydroxyhexadeca-*trans*-4-enoate (VI) was then obtained by condensation with zinc and ethyl bromoacetate

in tetrahydrofuran and was dehydrated with phosphoryl chloride in pyridine to give the ester from which the desired hexadeca-*trans*-2,*trans*-4-dienoic acid (VIII) was obtained on hydrolysis.

$$C_{11}H_{23}-C\equiv CH \xrightarrow[CH(OC_2H_5)_3]{C_2H_5MgBr} C_{11}H_{23}-C\equiv C\cdot CH(OC_2H_5)_2$$

(II) (III)

$$\xrightarrow[\text{Lindlar}]{H_2} C_{11}H_{23}CH=CH\cdot CH(OC_2H_5)_2 \xrightarrow{H^+} C_{11}H_{23}\cdot CH=CH\cdot CHO$$

*c.* *t.*

(IV) (V)

$$\xrightarrow[Zn]{BrCH_2CO_2Et} C_{11}H_{23}\cdot CH=CH\cdot CH\cdot CH_2\cdot CO_2Et$$

*t.* OH

(VI)

$$\xrightarrow{POCl_3,py} C_{11}H_{23}\cdot CH=CH\cdot CH=CH\cdot CO_2Et \xrightarrow{OH^-} \text{VIII}$$

*t.* *t.*

(VII)

### (*b*) *Hexadeca*-trans-*2*,cis-*4-dienoic Acid*

Hydrolysis of 1,1-diethoxytetradec-2-yne (III) gave tetradec-2-ynal (IX) which condensed readily with ethyl bromoacetate and zinc wool in tetrahydrofuran. The resulting hydroxy ester (X) dehydrated partially during distillation, but completely with phosphoryl chloride in pyridine. Hydrolysis of the ester afforded hexadeca-*trans*-2-en-4-ynoic acid (XI) from which hexadeca-*trans*-2,*cis*-4-dienoic acid (XII) was obtained by partial reduction.

$$C_{11}H_{23}\cdot C\equiv C\cdot CH(OEt)_2 \xrightarrow{H^+} C_{11}H_{23}\cdot C\equiv C\cdot CHO$$

(III) (IX)

$$\downarrow BrCH_2CO_2Et,\ Zn$$

$$C_{11}H_{23}\cdot C\equiv C\cdot CH=CH\cdot CO_2H \xleftarrow[OH^-]{(1)\ (2)\ POCl_3,py} C_{11}H_{23}\cdot C\equiv C\cdot CH\cdot CH_2CO_2Et$$

*t.* OH

(XI) (X)

$$\downarrow H_2$$

$$C_{11}H_{23}\cdot CH=CH\cdot CH=CH\cdot CO_2H$$

*c.* *t.*

(XII)

### (*c*) *Hexadeca*-cis-*2*,trans-*4-dienoic Acid*

This isomer was prepared by partial reduction of hexadeca-*trans*-4-en-2-ynoic acid (XX) but the preparation of this latter acid presented many difficulties. The first method attempted involved the condensation of tridecanal with acetylene monomagnesium bromide, a valuable reagent now readily available (Jones, Skattebøl, and Whiting 1956). Despite the ready polymerization of the long-chain aldehyde the acetylenic carbinol (XIII) was formed in reasonable yield. The tetrahydropyranyl derivative of XIII was converted to its bromomagnesium

salt and carbonated with solid carbon dioxide in an autoclave. Addition of ethanolic sulphuric acid to the reaction mixture esterified the acid and liberated the hydroxyl group giving ethyl 4-hydroxyhexadec-2-ynoate (XIV). The apparent inertia of this substance to dehydrating agents led to the suspicion that rearrangement to the ethoxy vinyl lactone (XV) had occurred (see Raphael 1955). However, the infra-red spectrum showed strong bands at 1714 (C=O), 2240 (C≡C), and 751 $cm^{-1}$ ($C{\equiv}C{\cdot}CO_2C_2H_5$) identifying the compound as the hydroxy-acetylenic ester (XIV).

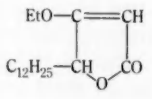
$$C_{12}H_{25}{\cdot}CHO + BrMg{\cdot}C{\equiv}CH \longrightarrow C_{12}H_{25}{\cdot}\underset{\displaystyle OH}{\underset{|}{CH}}{\cdot}C{\equiv}CH$$

(XIII)

$$\xrightarrow[(2)\ \ EtMgBr]{(1)\ Dihydropyran} C_{12}H_{25}{\cdot}\underset{\displaystyle O{\cdot}T}{\underset{|}{CH}}{\cdot}C{\equiv}C{\cdot}MgBr \xrightarrow[(2)\ EtOH,H^+]{(1)\ \ CO_2} C_{12}H_{25}{\cdot}\underset{\displaystyle OH}{\underset{|}{CH}}{\cdot}C{\equiv}C{\cdot}CO_2Et$$

T = Tetrahydropyranyl

(XIV)



(XV)

None of the usual methods of dehydration employing phosphoryl chloride in pyridine, potassium bisulphate, phosphorus pentoxide, and anhydrous copper sulphate, gave rise to enyne ester as judged by spectroscopic evidence.

Similarly, the acetylenic carbinol (XIII) could not be dehydrated directly, nor could the bromo or iodo derivative be dehydrohalogenated with potassium *tert.*-butoxide or diethylaniline at high temperatures. With sodamide in liquid ammonia, 3-bromopentadec-1-yne (XVI) was converted to 3-aminopentadec-1-yne (XVII). Attempted deamination by pyrolysis of the phosphate (Harries 1901) regenerated the amine. This could be readily converted to a quaternary methiodide in acetone with methyl iodide and potassium carbonate. Aqueous alkaline treatment of the quaternary iodide was unpromising but lithium butyl in ether under reflux gave indications of a reasonable yield of pentadec-3-en-1-yne (XVIII). The success of a parallel preparation utilizing the Wittig reaction caused abandonment of the deamination method.

$$C_{12}H_{25}{\cdot}\underset{\displaystyle Br}{\underset{|}{CH}}{\cdot}C{\equiv}CH \xrightarrow{NaNH_2} C_{12}H_{25}{\cdot}\underset{\displaystyle NH_2}{\underset{|}{CH}}{\cdot}C{\equiv}CH$$

(XVI) (XVII)

$$\xrightarrow{CH_3I+K_2CO_3} C_{12}H_{25}{\cdot}\underset{\displaystyle I^-N^+(CH_3)_3}{\underset{|}{CH}}{\cdot}C{\equiv}CH \xrightarrow{LiC_4H_9} C_{11}H_{23}{\cdot}CH{=}CH{\cdot}C{\equiv}CH$$

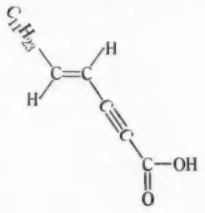
(XVIII)

Lauryl bromide and triphenyl phosphine would not form the quaternary phosphonium bromide (XIX) under " normal " conditions, in benzene at 0 °C or room temperature (Wittig and Sköllkopf 1954). In order to obtain a good yield it was necessary to heat the reactants to 140 °C in benzonitrile. After treatment with lithium butyl followed by propargyl aldehyde, pentadecenyne (XVIII) was obtained. The strong infra-red bands at 3318 (≡C—H), 2108 (C≡C), 1690 and 1617 (HC=CH), and 955 $cm^{-1}$ (*trans*=C—H) and absorption in the ultraviolet region with maxima at 222·5 and 228 (inflex) mμ (ε 12,200; 10,200) confirmed the structure of this hydrocarbon.

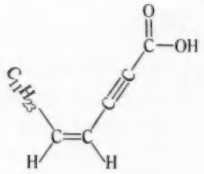
$$C_{12}H_{25}Br + P(Ph)_3 \longrightarrow \underset{\text{(XIX)}}{C_{12}H_{25}\cdot P^+(Ph)_3\cdot Br^-}$$

$$\overset{(1)}{(2)}\ \xrightarrow[OCH\cdot C\equiv CH]{LiC_4H_9} \underset{\text{(XVIII)}}{C_{11}H_{23}\cdot CH=CH\cdot C\equiv CH + O : P(Ph)_3}$$

Carboxylation of XVIII under pressure gave a mixture of acids from which the *trans*-acid (XX) was readily separated as its crystalline urea inclusion compound. It seems that the molecular shape of the *cis*-form (XXI) prevents formation of a similar complex.



(XX)
*trans*

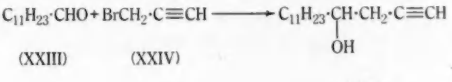


(XXI)
*cis*

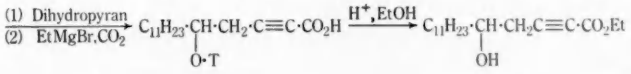
When urea was subsequently crystallized from the mother liquors only negligible amounts of acid were included so that by recovery of the *cis*-acid from the mother liquors almost a quantitative separation was possible. The proportion of *trans*-compound formed in the Wittig reaction was surprisingly small, the ratio of *trans*/*cis* being approximately 1/4. In previous reports of the Wittig reaction with propargyl aldehyde only the *trans*-isomer has been obtained in a yield of 40–50 per cent. and it has not been possible to isolate the *cis*-isomer (cf. Bohlmann and Viehe 1955).

Partial reduction of hexadeca-*trans*-4-en-2-ynoic acid gave the desired hexadeca-*cis*-2,*trans*-4-dienoic acid (XXII). This structure was confirmed through synthesis by an alternative route used by Eisner, Elvidge, and Linstead (1953) for the preparation of *cis*-2,*trans*-4-sorbic acid. Pentadec-1-yn-4-ol (XXV) was obtained from a Reformatsky reaction between dodecanal (XXIII) and propargyl bromide (XXIV) and after conversion into its tetrahydropyranyl ether, was carboxylated. Conversion of the hydroxyl group to the ether prevents formation of insoluble magnesium salts during carboxylation and leads to

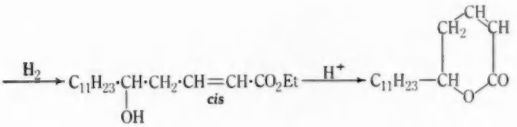
improved yields. Subsequent treatment with ethanolic sulphuric acid removed the tetrahydropyranyl group and also caused esterification. The resulting hydroxyacetylenic ester (XXVI) was partially reduced and the *cis*-olefinic ester was converted to the lactone (XXVIII) with boiling dilute hydrochloric acid. This lactone readily gave hexadeca-*cis*-2,*trans*-4-dienoic acid (XXII) with methanolic sodium methoxide.



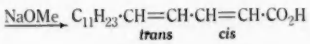
(XXIII) (XXIV) (XXV)



(XXVI)



(XXVII) (XXVIII)



(XXII)

### (*d*) *Hexadeca*-cis-*2*,cis-*4-dienoic Acid*

This isomer was first obtained by partial reduction of the corresponding diacetylenic acid. Sodium diacetylide was prepared by the addition of 1,4-dichlorobut-2-yne (XXIX) to three equivalents of sodamide in liquid ammonia cooled to −70 °C and reaction between this and undecyl bromide was attempted. The relatively high-melting bromide immediately solidified at this temperature and remained to a large extent unreacted. It was possible to improve the yields somewhat by the use of lithium diacetylide and by addition of undecyl bromide in ether but even under these conditions they never rose above 12 per cent., as estimated from the amount of acid obtained after carboxylation. It proved impracticable to isolate the pentadecadiyne (XXX) at this stage so the whole mixture was carboxylated and afterwards was extracted with potassium carbonate solution to obtain the hexadeca-2,4-diynoic acid (XXXI) produced. Some 85 per cent. of the undecyl bromide added was recovered from the neutral fraction by distillation. The diynoic acid was surprisingly stable in air; partial

reduction proceeded smoothly in light petroleum and pure hexadeca-*cis*-2,*cis*-4-dienoic acid (XXXII) was obtained after two crystallizations from the same solvent.

$$HO\cdot CH_2\cdot C{\equiv}C\cdot CH_2OH \xrightarrow{SOCl_2,py} ClCH_2\cdot C{\equiv}C\cdot CH_2Cl$$
(XXIX)

$$\xrightarrow{3NaNH_2} HC{\equiv}C\cdot C{\equiv}C\cdot Na \xrightarrow{C_{11}H_{23}Br} C_{11}H_{23}\cdot C{\equiv}C\cdot C{\equiv}CH$$
(XXX)

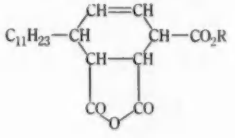
$$\xrightarrow{EtMgBr,CO_2} C_{11}H_{23}\cdot C{\equiv}C\cdot C{\equiv}C\cdot CO_2H \xrightarrow{2H_2} \text{XXXII}$$
(XXXI)

Alternatively, this isomer was readily prepared by partial reduction of hexadeca-*cis*-4-en-2-ynoic acid (XXI) obtained through the Wittig reaction discussed above. Both syntheses gave the same acid.

## III. Physical Properties

The longer alkyl group on the chromophore of these hexadecadienoic acids confers a stability greater than those of their $C_{10}$ and $C_6$-analogues. Generally speaking the compounds, once isolated, are more easily handled. Some decomposition still takes place in air but at a decreased rate. Over long periods in air the *trans-trans*-acid becomes coated with an insoluble solid polymeric material whereas under like circumstances the *cis-cis*-isomer becomes sticky. Stability increases from the acid through the ester to the *iso*butylamide.

The $C_{16}$-acids differ from the sorbic acids in that they do not deliquesce and they are all crystalline solids, unlike the decadienoic acids, of which only the *trans-trans* has been crystallized. As was found with the shorter chain compounds, the *trans-trans*-isomer was the only one to form a maleic anhydride adduct (XXXIII).



(XXXIII)

The relevant physical and optical properties of the acids and of their ethyl esters and *iso*butylamides are collected in Tables 1 and 2. In the shorter chain compounds of 6 and 10 carbon atoms the *cis-cis*-compounds possessed greater linearity than the *cis-trans*-isomers which led to tighter packing in the crystal and consequently a higher melting point. In the $C_{16}$ family the long chain has overcome the molecular symmetry effect of the chromophore and here the *cis-cis*-acid melts below the *cis-trans*-isomers.

The *iso*butylamides of the two *cis-trans*-compounds are anomalous in that they are both low-melting solids.

TABLE 1

PHYSICAL AND OPTICAL PROPERTIES OF ACIDS AND ESTERS

| Acid | Free Acid | | | Ethyl Ester | | | |
|---|---|---|---|---|---|---|---|
| | Melting Point | $\lambda_{max.}$ (mμ) | ε | Boiling Point | $n_D^{20}$ | $\lambda_{max.}$ (mμ) | ε |
| $C_{11}H_{23}CH{=}CHCH{=}CHCO_2H$ (*t.*) | 62·5–63·5 °C, 71·5–72 °C | 257 | 28,700 | 132–137 °C/ 0·1 mm | 1·4793 | 260·5 | 29,000 |
| $C_{11}H_{23}CH{=}CHCH{=}CHCO_2H$ (*c.*, *t.*) | 55·5–56·5 °C | 260 | 23,800 | 122–123 °C/ 0·1 mm | 1·4784 | 264 | 21,800 |
| $C_{11}H_{23}CH{=}CHCH{=}CHCO_2H$ (*t.*, *c.*) | 52–54 °C | 258 | 22,800 | 110–113 °C/ 0·1 mm | 1·4786 | 261·5 | 23,100 |
| $C_{11}H_{23}CH{=}CHCH{=}CHCO_2H$ (*c.*, *c.*) | 49–50 °C | 262 | 21,300 | 118–119 °C/ 0·1 mm | 1·4830 | 264·5 | 21,200 |
| $C_{11}H_{23}C{\equiv}CCH{=}CHCO_2H$ (*t.*) .. | 61–61·5 °C | 255 | 24,000 | 138–141 °C/ 0·3 mm | 1·4800 | 258 | 20,200 |
| $C_{11}H_{23}CH{=}CHC{\equiv}CCO_2H$ (*t.*) .. | 46·5–47·5 °C | 245·5 | 12,800 | 115 °C/ 0·1 mm | 1·4778 | 251 | 14,600 |
| | | 283* | | | | 283* | 2300 |
| $C_{11}H_{23}CH{=}CHC{\equiv}CCO_2H$ (*c.*) .. | 41–42 °C | 245 | 10,000 | 126–127 °C/ 0·2 mm | 1·4730 | 250·5 | 10,900 |
| | | 283* | 1200 | | | 283* | 1600 |
| $C_{11}H_{23}C{\equiv}CC{\equiv}CCO_2H$ .. | 63–64·5 °C | 222·5 | 1600 | — | — | — | — |
| | | 233·5 | 2000 | | | | |
| | | 245·5 | 3100 | | | | |
| | | 259 | 3900 | | | | |
| | | 274 | 2600 | | | | |

* Inflexion.

TABLE 2

PHYSICAL AND OPTICAL PROPERTIES OF *iso*BUTYLAMIDES

All ultraviolet absorption spectra were recorded in 95 per cent. ethanol on a Hilger Uvispek spectrophotometer

| *iso*Butylamide | Melting Point (°C) | $\lambda_{max.}$ (mμ) | ε |
|---|---|---|---|
| $C_{11}H_{23}{\cdot}CH{=}CH{\cdot}CH{=}CH{\cdot}CONHCH_2CH(CH_3)_2$ (*t.*, *t.*) | 91·5–93 | 258 | 33,000 |
| $C_{11}H_{23}{\cdot}CH{=}CH{\cdot}CH{=}CH{\cdot}CONHCH_2CH(CH_3)_2$ (*c.*, *t.*) | 26–29 | 261·5 | 28,600 |
| $C_{11}H_{23}{\cdot}CH{=}CH{\cdot}CH{=}CH{\cdot}CONHCH_2CH(CH_3)_2$ (*t.*, *c.*) | 30–32 | 259 | 27,600 |
| $C_{11}H_{23}CH{=}CH{\cdot}CH{=}CH{\cdot}CONHCH_2CH(CH_3)_2$ (*c.*, *c.*) | 67–68 | 261·5 | 24,800 |
| $C_{11}H_{23}{\cdot}C{\equiv}C{\cdot}CH{=}CH{\cdot}CONHCH_2CH(CH_3)_2$ (*t.*) .. | 83–85 | 256 | 25,000 |
| $C_{11}H_{23}{\cdot}CH{=}CH{\cdot}C{\equiv}C{\cdot}CONHCH_2CH(CH_3)_2$ (*t.*) .. | 45–46 | 249·5 | 15,000 |
| $C_{11}H_{23}{\cdot}CH{=}CH{\cdot}C{\equiv}C{\cdot}CONHCH_2CH(CH_3)_2$ (*c.*) .. | 32·5–33·5 | 248·5 | 12,000 |

## IV. Ultraviolet Absorption Spectra

The data shown in Tables 1 and 2 agree fairly well with those recorded for the shorter chain analogues. The four dienes show a single broad maximum with the *trans-trans*-isomer absorbing at the shortest wavelength and displaying the highest intensity. As the *trans*-bonds are replaced by *cis* the absorption moves towards longer wavelengths and lower intensities. There is a marked difference in the extinction coefficients at the bond maxima. The smaller values observed with the *cis-trans* and particularly the *cis-cis*-isomer have been attributed either to repulsion of substituents on the *cis*-bonds causing non-planarity, or to a decrease in the chromophore length. The effects of these factors on the chromophores under discussion have been adequately dealt with by Crombie (1955*c*) and will not be repeated here.

Table 3

INFRA-RED DATA ON 2,4-DIENOIC ACID DERIVATIVES

sh, shoulder ; s, strong ; m, medium ; w, weak band ; st, stretching ; be, bending vibration

| 2,4-Dienoic Acid Derivatives | C=O st | C=C st | =C−H *t*.be | C=C−COX *c*. | C=C−COX* *t*. | N−H |
|---|---|---|---|---|---|---|
| Ethyl hexadecadienoates (liq. film) | | | | | | |
| $C_{11}H_{23}CH{=}CHCH{=}CHCO_2C_2H_5$ (*t*. *t*.) | 1719s | 1649s, 1624m | 1002s 994sh, 961sh, 952w | — | 872m | |
| $C_{11}H_{23}CH{=}CHCH{=}CHCO_2C_2H_5$ (*c*. *t*.) | 1719s | 1642m, 1610w | 994m, 960w | — | 868m | |
| $C_{11}H_{23}CH{=}CHCH{=}CHCO_2C_2H_5$ (*t*. *c*.) | 1717s | 1643s, 1606s | 1002m, 964m | 821m | — | |
| $C_{11}H_{23}CH{=}CHCH{=}CHCO_2C_2H_5$ (*c*. *c*.) | 1717s | 1633m, 1594w | — | 825m | — | |
| Hexadecadienoic acids (" Nujol ") | | | | | | |
| $C_{11}H_{23}CH{=}CHCH{=}CHCO_2H$ (*t*. *t*.) | 1688s | 1638m, 1618m | 1012w 996m, 958w, 950w | — | 878m | |
| $C_{11}H_{23}CH{=}CHCH{=}CHCO_2H$ (*c*. *t*.) | 1693s | 1631m, 1610w | 1003w, 997w, 965w | — | 880w | |
| $C_{11}H_{23}CH{=}CHCH{=}CHCO_2H$ (*t*. *c*.) | 1691s | 1634m, 1603m | 1002w, 963w | 823w | — | |
| $C_{11}H_{23}CH{=}CHCH{=}CHCO_2H$ (*c*. *c*.) | 1694s | 1628m, 1594w | — | 832w | — | |
| *N-iso*Butyl hexadecadienamides (" Nujol ") | | | | | | |
| $C_{11}H_{23}CH{=}CHCH{=}CHCONHCH_2CH(CH_3)_2$ (*t*. *t*.) | 1628s | 1658s, 1615s | 998s, 986w | — | 875w | 3308m, 1550m |
| $C_{11}H_{23}CH{=}CHCH{=}CHCONHCH_2CH(CH_3)_2$ (*c*. *t*.) | 1625s | 1658s 1612sh | 993m, 957m | — | 869w | 3290m, 1552m |
| $C_{11}H_{23}CH{=}CHCH{=}CHCONHCH_2CH(CH_3)_2$ (*t*. *c*.) | 1630m | 1652s, 1607m | 998m, 962m | 815w | — | 3310m, 1550m |
| $C_{11}H_{23}CH{=}CHCH{=}CHCONHCH_2CH(CH_3)_2$ (*c*. *c*.) | 1627s | 1645m, 1600w | — | 834w 819w | — | 3348m, 1542m |

* Unconfirmed assignment.

## V. Infra-Red Spectra

The frequency values for the relevant bands of each spectrum are shown in Tables 3 and 4. They were recorded on a Perkin-Elmer Model 21 double-beam spectrometer fitted with a sodium chloride prism and are grouped so as to indicate

the effects of geometrical isomerism on the positions of maximal absorption. The values may be compared with those from an extensive list of spectra of conjugated ethylenic and acetylenic systems published by Allan, Meakins, and Whiting (1955) and also with corresponding figures for the $C_{10}$-compounds (Crombie 1955*c*). The main information to be gained from these figures is summarized below.

TABLE 4

INFRA-RED DATA ON ACETYLENIC COMPOUNDS

sh, shoulder ; s, strong ; m, medium ; w, weak band ; st, stretching ; be, bending vibration

| Acetylenic Compounds | C=O st | C=C st | =C−H *t.*be | C=C·COX* *t.* | C≡C·COX | C≡C st | N−H |
|---|---|---|---|---|---|---|---|
| **Ethyl esters (liq. film)** | | | | | | | |
| $C_{11}H_{23}\cdot C{\equiv}C\cdot \underset{t.}{CH{=}CH}\cdot CO_2C_2H_5$ | 1724s | 1623s | 962s | 860m | — | 2218m | |
| $C_{11}H_{23}\cdot \underset{t.}{CH{=}CH}\cdot C{\equiv}C\cdot CO_2C_2H_5$ | 1716s | 1632w | 961m | — | 750m | 2226s | |
| $C_{11}H_{23}\cdot \underset{c.}{CH{=}CH}\cdot C{\equiv}C\cdot CO_2C_2H_5$ | 1715s | 1616w | — | — | 747m | 2220s | |
| $C_{11}H_{23}\cdot C{\equiv}C\cdot C{\equiv}C\cdot CO_2C_2H_5$ | 1713s | — | — | — | 744m | 2242s, 2160w | |
| $C_{11}H_{23}\cdot C{\equiv}C\cdot \underset{OH}{CH}\cdot CH_2\cdot CO_2C_2H_5$ | 1743s | — | — | — | — | 2235w | |
| $C_{11}H_{23}\cdot CH_2\cdot \underset{OH}{CH}\cdot C{\equiv}C\cdot CO_2C_2H_5$ | 1714s | — | — | — | 751m | 2240m | |
| **Acids (" Nujol ")** | | | | | | | |
| $C_{11}H_{23}\cdot C{\equiv}C\cdot \underset{t.}{CH{=}CH}\cdot CO_2H$ | 1695s | 1623m | 962m | 864m | — | 2220m | |
| $C_{11}H_{23}\cdot \underset{t.}{CH{=}CH}\cdot C{\equiv}C\cdot CO_2H$ | 1680s | 1628w | 957m | — | 746w | 2236m, 2202s | |
| $C_{11}H_{23}\cdot \underset{c.}{CH{=}CH}\cdot C{\equiv}C\cdot CO_2H$ | 1684s | 1613w | — | — | 748w, 740w | 2218s | |
| $C_{11}H_{22}\cdot C{\equiv}C\cdot C{\equiv}C\cdot CO_2H$ | 1688s | — | — | — | 743m | 2244s, 2160w | |
| ***iso*Butylamides (" Nujol ")** | | | | | | | |
| $C_{11}H_{23}\cdot C{\equiv}C\cdot \underset{t.}{CH{=}CH}\cdot CONHCH_2CH(CH_3)_2$ | 1646s | 1618s | 969m | 860w | — | 2220w | 3330m, 1555m |
| $C_{11}H_{23}\cdot \underset{t.}{CH{=}CH}\cdot C{\equiv}C\cdot CONHCH_2CH(CH_3)_2$ | 1625s | — | 958m | — | — | 2218m | 3300m, 1548m |
| $C_{11}H_{23}\cdot \underset{c.}{CH{=}CH}\cdot C{\equiv}C\cdot CONHCH_2CH(CH_3)_2$ | 1622s | 1638s | — | — | 735sh | 2210m | 3280m, 1544m |

* Unconfirmed assignment.

*C=O Stretching.*—For a saturated ester this band occurs at about 1740 $cm^{-1}$ but is shifted to lower frequencies by conjugation. Similarly for saturated acids and *iso*butylamides, the carbonyl vibrations occurring at 1710 and 1642 $cm^{-1}$ respectively are each lowered by conjugation. In every case the position of the band is fairly independent of the configuration of the olefinic bond.

When a triple bond replaces the double bond in the 2-position there is apparently a greater shift towards lower frequencies, while for a corresponding replacement in the 4-position the shift is less. This supports the observations of Allan, Meakins, and Whiting (1955) but the results are not really conclusive as many of the differences are within the allowable error for the instrument.

*C=C Stretching.*—Two bands are present between 1660 and 1590 $cm^{-1}$ in the spectra of all the dienoic acids and derivatives. Both bands move to lower frequencies on passing from *trans-trans* to *trans-cis* to *cis-cis* but the band at lower frequency is the more affected. These shifts generally are pronounced and characteristic of each isomer.

When one triple bond is present there is only one band which appears at considerably lower frequency in the *cis-* than in the *trans*-isomer.

*Olefinic C—H Out-of-Plane Bending.*—With the exception of the *cis-cis*-isomer all of the dienes show a band around 960 $cm^{-1}$, although in the *trans-trans* this is merely a shoulder on a stronger band at 952 $cm^{-1}$. In addition every isomer containing a *trans*-double bond shows a sharp band at about 1000 $cm^{-1}$. *cis*-Bonds are more difficult to detect except when conjugated with the carbonyl of the acid function. When this happens a band appears at 820 $cm^{-1}$ (Sinclair *et al.* 1952 ; Crombie 1952*b*). It is interesting to note that in every compound in which a *trans*-double bond is conjugated with the acid carbonyl an isolated band of medium intensity is present around 870 $cm^{-1}$, which is lowered to 860 $cm^{-1}$ when the bond in the 4-position is acetylenic. There appears to be no previous reference to this band which seems quite characteristic in the compounds under discussion.

*C≡C Bands.*—Conjugation of an acetylene causes an increase in the intensity of the band at about 2220 $cm^{-1}$ (C≡C stretching) but little change in frequency and again these observations agree with those of Allan, Meakins, and Whiting (1955). The medium band at 750 $cm^{-1}$ is also present in the $C_{16}$-analogues. It was attributed by these authors to conjugation of acetylene and the carboxyl group of an acid or acid derivative.

## VI. Insecticidal Activities

The *iso*butylamides were tested for insecticidal activity by Mr. R. W. Kerr of the Division of Entomology, C.S.I.R.O. Two series of tests were carried out. In one series, batches of 10 female houseflies 5–6 days old were dosed on the dorsal surface of the thorax with $1 \times 10^{-4}$ ml of 5 per cent. solutions of the compounds in a 1 : 1 mixture of deodorized kerosene and acetone. In the other series, flies were exposed continuously for 2 days in vials coated inside with the dry powdered compounds. No indication of a stimulating, depressing, or toxic effect was seen in any of the tests.

It is clear then that the *iso*butylamides of the $C_{16}$ 2,4-dienoic acids lack insecticidal activity and that the activities of the naturally occurring *iso*butylamides must be attributed to some additional molecular feature. This conclusion agrees with that reached by Crombie from the examination of the corresponding $C_{10}$ family. It is proposed to introduce the additional molecular features that appear necessary.

## VII. Experimental

Microanalyses were carried out in the C.S.I.R.O. Microanalytical Laboratory at the University of Melbourne. Melting points were observed on a Kofler heating stage microscope and are corrected ; boiling points are uncorrected. All distillations and evaporations were carried out under nitrogen. Infra-red measurements were made by Mr. A. Triffett using a Perkin-Elmer Model 21 double-beam spectrometer with sodium chloride prism.

*General Methods.—Partial Reduction of Acetylenic Compounds.* The general method is similar to that used by Allan, Jones, and Whiting (1955). The acetylenic compound, in methyl acetate or light petroleum, was shaken in hydrogen with Lindlar's catalyst and quinoline (30–40 g of catalyst and 10 g of quinoline for each acetylene gramme formula weight). Sometimes exactly 1 mol of hydrogen was absorbed but with an occasional batch of catalyst it was necessary to supply extra to complete reduction to the olefin. The catalyst was filtered off and the filtrate shaken with dilute sulphuric acid to remove quinoline, washed with water, dried ($Na_2SO_4$), and evaporated. The crude product was purified by distillation or crystallization.

It should be mentioned that the use of Analar light petroleum as solvent gave somewhat erratic results, some batches being quite satisfactory while with others the reduction would not proceed at all.

*Preparation of Esters.* Conversion of the acids to ethyl esters where required was accomplished by dissolving the acid in absolute ethanol containing 4–5% concentrated sulphuric acid and setting aside at room temperature for 48 hr. After addition of water the ester was extracted with ether and distilled.

*Preparation of* iso*Butylamides.* (i) In most cases the acid was converted to the acid chloride by warming in ether or benzene for 1 hr with a slight excess of oxalyl chloride, after which the solvent was removed under reduced pressure. Removal of the last traces of oxalyl chloride was difficult but essential as the di-*iso*butylamide of oxalic acid which resulted on subsequent addition of *iso*butylamine in ether was very difficult to remove. Warming on the steam-bath under high vacuum was generally sufficient to remove all of the oxalyl chloride.

(ii) In some cases which are indicated subsequently a more elaborate method was employed. The monomagnesium bromide of *iso*butylamine was prepared in the cold and treated with the ethyl ester of the appropriate acid. After several hours at room temperature, dilute mineral acid was added to give the desired *iso*butylamide generally in good yield.

(*a*) *1,1-Diethoxytetradec-2-yne.*—1-Tridecyne (95 g), prepared from undecyl bromide and sodium acetylide (Lumb and Smith 1952) was added slowly as an ethereal solution to ethylmagnesium bromide from magnesium (13 g) and ethyl bromide (63 g) in ether (150 ml). The solution was stirred and refluxed until evolution of ethane had ceased (30 min), after which the solution was cooled in ice and ethyl orthoformate (87 g) in ether (100 ml) added during 30 min. Overnight the mixture was stirred and then refluxed for 5 hr, again cooled, and dilute acetic acid added carefully. The ethereal layer was separated, washed with bicarbonate and with water, and dried ($Na_2SO_4$). Removal of the ether and fractionation of the residue through a packed column of glass helices gave the acetal as a colourless liquid (109 g), b.p. 114–116 °C/0·2 mm, $n_D^{20}$ 1·4470.

(*b*) *Tetradec-2-ynal.*—The above acetal (42 g) was heated under reflux in nitrogen with 4% aqueous sulphuric acid (100 ml) for 4 hr. The top layer was separated with ether, washed, and distilled to give tetradecynal (15·2 g), b.p. 94–98 °C/0·3 mm, $n_D^{18}$ 1·4590. The 2,4-dinitrophenylhydrazone formed bright yellow plates, m.p. 32–33 °C (Found: C, 61·7; H, 7·2; N, 14·4%. Calc. for $C_{20}H_{28}N_4O_4$: C, 61·8; H, 7·3; N, 14·4%).

In later experiments it was found unnecessary to fractionate the acetal; as pure a material was obtained if the crude acetal was hydrolysed with sulphuric acid and the aldehyde isolated through the bisulphite compound.

(*c*) *Ethyl 3-Hydroxyhexadec-4-ynoate.*—Zinc wool (5·4 g) was cleaned by treatment with dilute sulphuric acid, washed with water and acetone, dried at 100 °C for a short time, and covered with tetrahydrofuran (10 ml). A little mercuric chloride was added and the solvent refluxed during the gradual addition of ethyl bromoacetate (12·6 g) and tetradecynal (15·2 g) in tetrahydrofuran. After heating for a further 2 hr very little zinc remained; ice and acetic acid were added and the product isolated with ether. Distillation gave the hydroxy ester (9·6 g), b.p. 155–157 °C/0·5 mm, $n_D^{22}$ 1·4613 (Found: C, 73·4; H, 10·8%. Calc. for $C_{18}H_{32}O_3$: C, 72·9; H, 10·9%). The compound was probably contaminated with some of the dehydrated material, formed during distillation.

Hydrolysis with potassium hydroxide in methanol gave the hydroxy acid as white prisms, m.p. 75·5–76·5 °C (Found : C, 71·8 ; H, 10·5%. Calc. for $C_{16}H_{28}O_3$ : C, 71·6 ; H, 10·5%).

(*d*) *Ethyl Hexadeca*-trans-*2-en-4-ynoate*.—The hydroxy ester (3·95 g) in pyridine (4 ml) was mixed while cooling in ice with phosphoryl chloride (4·7 ml) in pyridine (12 ml). After heating at 100 °C for 3 hr under nitrogen dilute sulphuric acid and ether were added and the ethereal layer washed with dilute acid and water to remove pyridine. After drying ($Na_2SO_4$) and removal of the ether followed by distillation the enyne ester (2·1 g) was obtained as a pale yellow viscous oil, b.p. 138–141 °C/0·3 mm, $n_D^{20}$ 1·4800 (Found : C, 77·5 ; H, 10·8%. Calc. for $C_{18}H_{30}O_2$ : C, 77·7 ; H, 10·9%).

(*e*) *Hexadeca*-trans-*2-en-4-ynoic Acid*.—The ethyl ester (1·0 g), methanol (5 ml), and potassium hydroxide (0·26 g) were refluxed for 1 hr under nitrogen. Water and dilute sulphuric acid were added and the product extracted with ether. Crystallization from acetonitrile gave colourless plates (0·6 g), m.p. 61–61·5 °C (Found : C, 77·1 ; H, 10·4%. Calc. for $C_{16}H_{26}O_2$ : C, 76·8 ; H, 10·5%). The *iso*butylamide crystallized from light petroleum as white plates, m.p. 83–85 °C (Found : C, 78·4 ; H, 11·3 ; N, 4·8%. Calc. for $C_{20}H_{35}NO$ : C, 78·6 ; H, 11·6 ; N, 4·6%).

(*f*) *Hexadeca*-trans-*2*,cis-*4-dienoic Acid*.—Hexadeca-*trans*-2-en-4-ynoic acid (0·3 g) in methyl acetate (20 ml) was hydrogenated in the presence of Lindlar's catalyst (40 mg) and quinoline (13 mg). 1·0 mol of hydrogen was absorbed. The residue, after filtration and evaporation of the solvent, when crystallized from aqueous acetic acid formed colourless plates (0·11 g), m.p. 55·5–56·5 °C (Found : C, 76·1 ; H, 11·2%. Calc. for $C_{16}H_{28}O_2$ : C, 76·1 ; H, 11·2%).

The same acid was obtained by partial reduction of ethyl hexadeca-*trans*-2-en-4-ynoate, followed by hydrolysis. Ethyl hexadeca-*trans*-2,*cis*-4-dienoate was also prepared from the acid with cold ethanolic sulphuric acid. It had b.p. 122–123 °C/0·2 mm, $n_D^{20}$ 1·4784 (Found : C, 77·3 ; H, 11·6%. Calc. for $C_{18}H_{32}O_2$ : C, 77·1 ; H, 11·5%). The *iso*butylamide was a low-melting solid, m.p. 27–29 °C, whether prepared by either method (i) or (ii). It could be crystallized from light petroleum or acetone at —40 °C. A material of satisfactory purity was obtained by chromatography on neutral alumina. The later fractions always contained some of the *trans-trans*-isomer (Found : C, 77·7 ; H, 12·2 ; N, 4·4%. Calc. for $C_{20}H_{37}NO$ : C, 78·1 ; H, 12·1 ; N, 4·6%).

(*g*) *1,1-Diethoxytetradeca*-cis-*2-ene*.—1,1-Diethoxytetradec-2-yne (50 g) in methyl acetate was partially hydrogenated in the presence of Lindlar's catalyst (2 g) and quinoline (0·6 ml). Absorption of gas was slow until a further 3 g of catalyst was added. After the calculated volume had been absorbed the catalyst was removed and the solution worked up by the general procedure. Distillation gave the olefinic acetal (49 g), b.p. 90–93 °C/0·3 mm, $n_D^{18}$ 1·4430 (Found : C, 75·9 ; H, 12·5%. Calc. for $C_{18}H_{36}O_2$ : C, 76·0 ; H, 12·8%).

(*h*) *Tetradeca*-trans-*2-enal*.—The acetal (49 g) was hydrolysed in boiling 5% aqueous sulphuric acid (300 ml) under nitrogen for 3 hr. Distillation after washing gave tetradecenal (31 g), b.p. 84–86 °C/0·1 mm, $n_D^{20}$ 1·4584. The 2,4-dinitrophenylhydrazone formed orange needles, m.p. 126–127 °C (Found : C, 61·5 ; H, 7·7 ; N, 14·1%. Calc. for $C_{20}H_{30}N_4O_4$ : C, 61·5 ; H, 7·7 ; N, 14·4%).

(*i*) *Ethyl 3-Hydroxyhexadeca*-trans-*4-enoate*.—A Reformatsky reaction was carried out as described above for the acetylenic aldehyde, using zinc wool (5·4 g), tetradecenal (15 g), and ethyl bromoacetate (12·6 g) in tetrahydrofuran. Isolation gave the hydroxy ester which distilled (11·7 g) without signs of dehydration, b.p. 154–158 °C/0·6 mm, $n_D^{17}$ 1·4590 (Found : C, 72·4 ; H, 11·5. Calc. for $C_{18}H_{34}O_3$ : C, 72·4 ; H, 11·5%).

(*j*) *Ethyl Hexadeca*-trans-*2*,trans-*4-dienoate*.—Ethyl 3-hydroxyhexadeca-*trans*-4-enoate (5 g) in pyridine (5 ml) was added to phosphoryl chloride (5·95 ml) in pyridine (15 ml) with cooling and the mixture was afterwards heated at 100 °C for 3 hr under nitrogen. The products when worked up as described for the acetylenic ester above and distilled gave 3 g of the diene ester as a colourless liquid, b.p. 132–137 °C/0·1 mm, $n_D^{20}$ 1·4793 (Found : C, 77·3 ; H, 11·7%. Calc. for $C_{18}H_{32}O_2$ : C, 77·1 ; H, 11·5%). The ester combined with maleic anhydride when heated in

benzene in a sealed tube at 100 °C for 1 hr. The adduct crystallized from light petroleum as large colourless prisms, m.p. 74–76 °C (Found : C, 69·8 ; H, 8·9%. Calc. for $C_{22}H_{34}O_5$ : C, 69·8 ; H, 9·1%).

(*k*) *Hexadeca*-trans-*2*,trans-*4-dienoic Acid*.—The above ester (3 g) was hydrolysed by refluxing with potassium hydroxide (0·76 g) in methanol (15 ml) for 75 min under nitrogen. Crystallization of the product from acetonitrile and then from light petroleum (b.p. <40 °C) gave the dienoic acid (1·9 g) as flat, white prisms which melted first at 62·5–63·5 °C, resolidifying to melt finally at 71·5–72 °C (Found : C, 76·2 ; H, 11·2%). The *iso*butylamide crystallized from light petroleum in white needles, m.p. 91·5–93 °C (Found : C, 77·9 ; H, 12·1 ; N, 4·7%).

(*l*) *Pentadec-1-yn-3-ol*.—Ethynylmagnesiumbromide was prepared according to the method of Jones, Skatteböl, and Whiting (1956). Ethyl magnesium bromide prepared in tetrahydrofuran from magnesium (12 g) and ethyl bromide (60 g) was added while still warm in 2–3 ml portions to tetrahydrofuran (100 ml) saturated with acetylene and with acetylene bubbling through rapidly. To this solution tridecanal (70 g ; Lauer, Gensler, and Miller 1941) was added and the mixture stirred overnight at room temperature. Saturated ammonium chloride solution was added and the pentadecynol extracted with ether and distilled. There was obtained 53 g of a white solid, b.p. 114–118 °C/0·3 mm, m.p. 33–36 °C (Found : C, 80·5 ; H, 12·8%. Calc. for $C_{15}H_{28}O$ : C, 80·3 ; H, 12·6%).

(*m*) *Ethyl 4-Hydroxyhexadec-2-ynoate*.—The pentadecynol (32 g) was converted to its tetrahydropyranyl ether by adding to dihydropyran (12·5 g) and phosphoryl chloride (0·1 ml). The mixture first became warm. After several hours it was poured into aqueous potassium hydroxide and extracted with ether. Since on attempted distillation there were signs of decomposition the ether was used without further purification. It was added to ethylmagnesium bromide (from 3·5 g Mg) in ether and after refluxing for 2 hr the solution was sealed in an autoclave with solid $CO_2$ and left for 24 hr. The mixture was transferred to a flask containing ethanol (500 ml) and concentrated sulphuric acid (25 g) and set aside for 48 hr. The resulting ester was worked up in the usual way and distilled. The oil obtained (30 g), from spectroscopic evidence, was substantially pure ethyl 4-hydroxyhexadec-2-ynoate, b.p. 150–156 °C/0·1 mm, $n_D^{20}$ 1·4672 (Found : C, 72·5 ; H, 11·0 ; active H, 0·36% at 95 °C. Calc. for the hydroxy ester, $C_{18}H_{32}O_3$ : C, 72·9 ; H, 10·9 ; active H, 0·34%).

(*n*) *3-Bromopentadec-1-yne*.—Pentadec-1-yn-3-ol (53 g) in dry ether (40 ml) and pyridine (2·4 ml) at —10 °C was brominated by slow addition of phosphorus tribromide (23·4 g) in ether (20 ml). After addition the cooling bath was removed and the mixture was stirred overnight. After washing with water and with potassium bicarbonate solution the product was distilled to give the bromide as a colourless oil (45 g), b.p. 105–111 °C/0·1–0·2 mm, $n_D^{20}$ 1·4698. The bromine content was low (Found : Br, 26·1%. Calc. for $C_{15}H_{27}Br$ : Br, 27·8%).

(*o*) *Treatment with Sodamide*.—3-Bromopentadec-1-yne (45 g) in ether (100 ml) was added slowly to sodamide prepared in liquid ammonia (500 ml) from sodium (11 g). Stirring was continued overnight, solid ammonium chloride (20 g) was added carefully, and the mixture tipped into a beaker and the ammonia allowed to evaporate. Water and ether were added to the residue, the ethereal layer was separated, and when washed thoroughly and distilled gave a low-melting solid which proved to be essentially 3-aminopentadec-1-yne, b.p. 91–95 °C/0·05 mm (Found : C, 81·0 ; H, 13·0 ; N, 4·7, 5·0%. Calc. for $C_{15}H_{29}N$ : C, 80·7 ; H, 13·1 ; N, 6·3%). The optical density at 223 mμ indicated that very little dehydrobromination had occurred. On treatment of the amine with phosphoric acid in aqueous ethanol the phosphate precipitated as long white needles, m.p. 113–116 °C. On distillation at 0·3 mm the amine was regenerated.

(*p*) *Hofmann Degradation*.—3-Aminopentadec-1-yne (1 g) was suspended in 25% sodium hydroxide solution (10 ml) at the boiling point and dimethyl sulphate (2 ml) added in small quantities over 15 min. A strong smell of trimethylamine was evident. After 30 min refluxing, water was added, and the product isolated by extraction with ether. The residue had $\lambda_{max.}$ 223 mμ (ε, 2640).

(*q*) *Trimethylpentadecynylammonium Iodide*.—3-Aminopentadec-1-yne (22·8 g) in acetone was refluxed with excess methyl iodide in the presence of solid potassium carbonate. An excess of methyl iodide was maintained by gradual addition over 15 hr refluxing time. After filtration

and evaporation the residue was extracted with chloroform to remove the methiodide but not potassium iodide. Addition of ether precipitated trimethylpentadecynylammonium iodide (31 g) as a white solid, m.p. 121 °C (Found : I, 32·4%. Calc. for $C_{18}H_{36}NI$ : I, 32·3%).

(*r*) *Treatment with Lithium Butyl.*—The quaternary iodide (15 g) was suspended in dry ether and an ethereal solution of lithium butyl (containing 1 equiv) was added with stirring and cooling. The solid rapidly dissolved and a red solution resulted. This was stirred for several hours at room temperature and for 2 hr at the boiling point, and then decomposed with water. From the ethereal layer after washing was obtained a colourless oil having $\lambda_{max.}$ 222·5 mμ (ε, 3100).

(*s*) *Lauryl Triphenylphosphonium Bromide.*—Lauryl bromide (48·7 g) and triphenylphosphine (56·4 g) in benzonitrile (150 ml) were heated in an oil-bath to 140–150 °C for 3 hr. About one-half of the solvent was then removed under reduced pressure and the remainder was diluted with much dry ether. An oil separated which solidified on further treatment with ether to give a white crystalline quaternary salt (81 g), m.p. 98–100 °C (Found : Br, 15·9%. Calc. for $C_{30}H_{40}BrP$ : Br, 15·6%).

(*t*) *Pentadec-3-en-1-yne.*—Lauryl triphenylphosphonium bromide (51 g) was suspended in dry ether and lithium butyl (1 equiv) in ether added slowly with cooling. After 2 hr stirring, a sticky yellow precipitate was present in a deep red solution. Propargyl aldehyde (7 g) in ether was then added with ice cooling and the resultant suspension was stirred overnight at room temperature and 1 hr at the boiling point. After cooling dilute sulphuric acid was added, the ethereal layer was separated, washed, and evaporated. Distillation of the residual oil gave pentadec-3-en-1-yne (11·8 g) as a colourless oil, b.p. 63–65 °C/0·05 mm, $n_D^{19}$ 1·4587 (light absorption : $\lambda_{max.}$ 222·5, 228 (inflex) mμ (ε, 12,200, 10,200)( Found : C, 87·3 ; H, 12·5%. Calc. for $C_{15}H_{26}$ : C, 87·3 ; H, 12·7%).

(*u*) *Hexadec-4-en-2-ynoic Acid.*—Pentadecenyne (10·4 g) in ether was added to ethylmagnesium bromide (from Mg 1·3 g) in ether and the solution refluxed until evolution of ethane had ceased (1 hr). The Grignard solution, in a 100 ml flask, was sealed into an autoclave with solid $CO_2$ for 20 hr. The mixture was decomposed by the addition of saturated ammonium chloride solution and the ethereal layer was separated and washed. Evaporation left a solid residue which was dissolved in warm methanol (100 ml) containing urea (30 g). After cooling the urea complex separated, and was washed with a little methanol saturated with urea. A second treatment of the methanolic mother liquors with urea produced negligible additional amounts of the complex. The complex was decomposed with very dilute sulphuric acid. The carboxylic acid liberated (1·5 g) was pure after one crystallization from light petroleum (b.p. <40 °C). The hexadeca-*trans*-4-en-2-ynoic acid melted at 46·5–47·5 °C (Found : C, 77·1 ; H, 10·8%). The ethyl ester had b.p. 115 °C/0·1 mm, $n_D^{20}$ 1·4778 (Found : C, 77·9 ; H, 11·1 ; O, 12·0%). The *iso*-butylamide was prepared from the ester and *iso*butylamine magnesium bromide ; it melted at 45–46 °C (Found : C, 78·9 ; H, 11·7 ; N, 4·3%).

The *cis*-isomer was obtained by dilution of the methanolic mother liquors with water and extraction with ether. It formed plates (6·3 g) from light petroleum (b.p. <40 °C) m.p. 41–42 °C (Found : C, 77·0 ; H, 10·6 ; O, 12·6%). The *cis*- and *trans*-isomers were quite distinct, a mixture had m.p. 32–40 °C. Ethyl hexadeca-*cis*-4-en-2-ynoate had b.p. 126–127 °C/0·2 mm, $n_D^{20}$ 1·4730 (Found : C, 78·0 ; H, 10·9%). The *iso*butylamide (Grignard method) had m.p. 32·5–33·5 °C (Found : C, 78·6 ; H, 11·5 ; N, 4·6%).

(*v*) *Hexadeca*-cis-2,trans-*4-dienoic Acid.*—(i) Hexadeca-*trans*-4-en-2-ynoic acid (0·5 g) in methyl acetate (10 ml) was partially hydrogenated in the presence of Lindlar's catalyst (0·1 g) and quinoline (0·02 g). It was necessary to add more catalyst during the reduction as absorption became very slow before reduction was complete. The hydrogenation was stopped after 90% of the theoretical volume had been absorbed, the catalyst was removed by filtration, and the quinoline by shaking with dilute sulphuric acid. The product was crystallized several times from light petroleum (b.p. <40 °C) to give the pure *cis*-2,*trans*-4-acid (0·21 g), m.p. 52–54 °C (Found : C, 76·2 ; H, 11·2%). The ethyl ester had b.p. 110–113 °C/0·1 mm, $n_D^{20}$ 1·4786 (Found : C, 77·4 ; H, 11·5%). The *iso*butylamide was obtained through the acid chloride and also by way of the ester. Both methods produced an oil or a low-melting solid purifiable by repeated crystallization to colourless prisms, m.p. 30–32 °C (Found : C, 78·4 ; H, 12·1 ; N, 4·8%).

(*w*) *Pentadec-1-yn-4-ol.*—Zinc wool (7·5 g), etched and dried as above, was covered with dry tetrahydrofuran (25 ml) and the solvent refluxed during the gradual addition of a mixture of dodecanal (21·2 g) and propargyl bromide (15 g) in tetrahydrofuran (25 ml). After heating for a further 1 hr the solution was cooled and poured into ice-cold dilute acetic acid and the product isolated with ether. Distillation gave the acetylenic carbinol as a white solid (9·65 g), b.p. 160–165 °C/0·2 mm, m.p. 38–39·5 °C (Found : C, 80·6 ; H, 12·5%).

(*x*) *5-Hydroxyhexadec-2-ynoic Acid.*—Pentadec-1-yn-4-ol (9·65 g) was converted to its tetrahydropyranyl ether with dihydropyran (3·65 g) and a trace of phosphoryl chloride. After standing for 3 hr at room temperature the ether was converted to its magnesium bromide with ethylmagnesium bromide (from 1·3 g Mg) and carboxylated under pressure. After 24 hr the acid was liberated with dilute sulphuric acid and extracted with ether. Evaporation of ether and crystallization from light petroleum gave the hydroxy acid (8·7 g) as white prisms, m.p. 57–58·5 °C (Found : C, 72·0 ; H, 10·6%).

(*y*) *Hexadec-2-eno-5-lactone.*—Ethyl 5-hydroxyhexadec-2-ynoate (7·1 g, $n_D^{20}$ 1·4650) was partially hydrogenated in the presence of Lindlar's catalyst by the general method. After removal of the catalyst and the quinoline ethyl 5-hydroxyhexadeca-*cis*-2-enoate (5·8 g) was obtained by evaporation of the solvent and distillation. It had b.p. 165 °C/0·4 mm, $n_D^{20}$ 1·4667. Without further purification the ester (4·65 g) was treated with boiling 2N hydrochloric acid (50 ml) under reflux for 80 min. On cooling, the lactone solidified and was isolated with ether and distilled ; b.p. 140–142 °C/0·1 mm, $n_D^{20}$ 1·4689, the distillate solidifying on cooling. Crystallization from methanol or light petroleum proved to be extravagant but afforded colourless plates of the lactone, m.p. 27–29 °C (Found : C, 76·5 ; H, 11·3%). The infra-red spectrum showed bands at 1723 (αβ-unsaturated δ-lactonic (C=O) and 815 $cm^{-1}$ (*cis*-C=C.COX).

(*z*) *Hexadeca-*cis*-2,*trans*-4-dienoic Acid.*—(i) Sodium (0·37 g) in dry methanol (34 ml) was cooled to 20 °C and added to a solution of the lactone (3·65 g) in methanol (3 ml). After 90 min at room temperature the solvent was evaporated under reduced pressure, the residue acidified, and extracted with ether. After washing, the ethereal solution was evaporated and the residue crystallized several times from light petroleum to give an acid (2·1 g) whose ultraviolet and infra-red spectra and melting point were identical with those of hexadeca-*cis*-2,*trans*-4-dienoic acid obtained above. A mixed melting point did not depress.

(*aa*) *Pentadeca-1,3-diyne.*—(i) Sodamide (from 13·8 g Na) was prepared in liquid ammonia (250 ml) and liquid nitrogen was poured into the vigorously stirred solution until the temperature had dropped to —70 °C. 1,4-Dichlorobut-2-yne (24·6 g) was then added very slowly followed after 5 min by undecyl bromide (47 g) which solidified immediately. The mixture was stirred for 16 hr, and then ether (200 ml) was added, followed by solid ammonium chloride (20 g). After 5 min stirring the whole was tipped into a beaker, covered with " Cellophane ", and set aside until the ammonia had evaporated. The residue was then extracted several times with dry ether, evaporation of which gave a brown oily residue absorbing in the ultraviolet at 219, 228, 238 (inflex), 253, 266, 281, and 298 mμ (ε, 406, 442, 420, 120, 175, 270, 275 respectively). As the bands at 228, 238, and 253 mμ are those relevant to a monosubstituted diacetylene it is obvious that a number of polyenynes are present ; for example, the bands at 266, 281, and 298 mμ are present in the spectrum of oenanthetol, which has an enediynediene chromophore. As these compounds absorb much more intensely than a diacetylene the amounts present would not be very great, probably of the order of 1%. Attempted distillation of the reaction product resulted in partial decomposition. The material was therefore used directly.

(ii) Lithium was substituted for the sodamide of experiment (i) and the ammonia was cooled externally in an ethanol–solid $CO_2$ bath to —75 °C before adding the dichlorobutyne. Addition of the undecyl bromide was made in ether to increase the solubility and when this was complete the reaction mixture was allowed to warm to —35 °C, and was stirred at this temperature overnight. Working up in the usual way gave an oily product whose ultraviolet absorption spectrum was similar to that from method (i) with slightly higher intensities.

(*bb*) *Hexadeca-2,4-diynoic Acid.*—The material from three such experiments was added to ethylmagnesium bromide (from 6 g Mg) in ether, and during 30 min heating, approx. 1600 ml of ethane was evolved. The products were transferred to an autoclave and sealed in the presence

of solid $CO_2$ for 48 hr. The diacetylenic acid produced was first liberated with dilute sulphuric acid and then extracted from the ethereal solution with dilute potassium carbonate solution. It crystallized from light petroleum (b.p. 40–60 °C) first as pale yellow microprisms (1·95 g), but when decolourized by treatment with charcoal in light petroleum and recrystallized it melted at 63–64·5 °C. The pale yellow compound was analytically satisfactory (Found: C, 77·3; H, 9·7%. Calc. for $C_{16}H_{24}O_2$: C, 77·4; H, 9·7%). Light absorption: $\lambda_{max.}$ 222·5, 233·5, 245·5, 259, 274 mμ (ε, 1570, 1970, 3065, 3875, 2640 respectively); $\lambda_{min.}$ 219, 228, 239, 252·5, 267·5 mμ (ε, 1490, 1240, 1260, 1380, 990 respectively). The first five minima correspond to the maxima of the pentadeca-1,3-diyne. Higher intensities were obtained for some fractions of the diacetylenic acid but the minima were also much higher, indicating the presence of impurities.

(cc) *Hexadeca*-cis-*2,*cis-*4-dienoic Acid.*—(i) The diynoic acid (0·5 g) in light petroleum containing quinoline (0·2 g) was shaken with hydrogen in the presence of Lindlar's catalyst (0·2 g) until 98 ml had been absorbed (theor. 93·5 ml at 18 °C/775 mm). The solution was filtered, washed with dilute sulphuric acid and water. Crystallization from light petroleum then gave the *cis-cis*-acid (0·26 g), m.p. 49–50 °C, as white plates (Found: C, 76·0; H, 11·2%). The ethyl ester had b.p. 118–119 °C/0·1 mm, $n_D^{20}$ 1·4830 (Found: C, 77·4; H, 11·4; O, 11·7%) and the *iso*butylamide (from the acyl chloride and amine) had m.p. 67–68 °C (Found: C, 78·0; H, 12·1; N, 4·7%).

(ii) Hexadeca-*cis*-4-en-2-ynoic acid (1·27 g) in methyl acetate (12 ml) and quinoline (0·05 g) was reduced in the presence of Lindlar's catalyst (0·15 g) until 1 mol-equiv. had been absorbed. Working up as above gave the *cis-cis*-acid (0·85 g) from light petroleum, identical in every respect with that obtained from the diynoic acid.

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# ACETYLENIC ACIDS FROM FATS OF SANTALACEAE AND OLACACEAE: SEED AND ROOT OILS OF *EXOCARPUS CUPRESSIFORMIS* LABILL.

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*Summary*

Octadec-*trans*-11-en-9-ynoic acid (ximenynic acid) forms over 60 per cent. of the acids present as glycerides in the seed fat of *Exocarpus cupressiformis* Labill. The roots of this tree contain an oil which is mainly a fat with octadec-*trans*-13-en-9,11-diynoic acid forming 59 per cent. of the fatty acids. These two acids are also major components respectively of the seed and root fats of the related species *E. stricta* R.Br. The root fat of *Ximenia americana* Linn. is rich in acetylenic acids.

## I. Introduction

Octadec-*trans*-11-en-9-ynoic acid (ximenynic acid) is a major component of the acids from the seed fats of several members of the *Ximenia* genus of the Olacaceae family (Ligthelm and Schwartz 1950; Ligthelm, Schwartz, and von Holdt 1952) and as well of several members of the *Santalum* genus of the Santalaceae (Gunstone and McGee 1954; Hatt and Szumer 1954; Hatt and Schoenfeld 1956). It was suggested earlier (Hatt and Szumer 1954) that this acid might also be present in seed fats of species belonging to other genera of the order Santalales to which these two families belong. In testing this suggestion further the first species chosen for examination was *Exocarpus cupressiformis* Labill. This tree is a member of a genus of some 17 species of the Santalaceae (Ewart 1930) and has the advantage that it is common and widely distributed in south-eastern Australia where it is known as the native cherry or cherry Ballart. Like the Australian members of the *Santalum* genus it is suspected to be parasitic on other plants. It is a small tree which attains a height of about 30 ft and is conspicuous for its cypress-like foliage and for its small ovoid nut which is attached to a swollen red stalk and ripens in Victoria from October to December.

The seed fat was readily extracted with light petroleum as a light yellow oil. The fatty acids from this oil contained 60 per cent. of ximenynic acid, which may be compared with the 36 to 45 per cent. of ximenynic acid present in the fatty acids from the seed fats of the three Australian species of the *Santalum* genus already examined (Hatt and Schoenfeld 1956), and with the 95 per cent. reported in the seed fat of the Indian species *Santalum album* Linn. (Gunstone and Russell 1955). This seed fat, like those from the three Australian species of *Santalum*, contained a small percentage of a rubbery material which was precipitated when the extracted fat was mixed with acetone.

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In correspondence Professor N. A. Sörensen had suggested that other portions of plants of the Santalales should be examined for their possible content of acetylenic acids. When acetone extracts were made of other parts of the native cherry tree, spectroscopic examination at once revealed the presence of other acetylenic materials in particularly high concentration in extracts of the root. Removal of the acetone from the root extract left an oil which deposited a dark material when kept for a time. If this dark impurity was filtered off a deep yellow oil remained (yields 1 to 1·5 per cent.), composed almost entirely of glycerides, and of these those of octadec-*trans*-13-en-9,11-diynoic acid (I) formed some 59 per cent.

$$CH_3(CH_2)_3CH{=}CH{-}C{\equiv}C{-}C{\equiv}C{-}(CH_2)_7COOH,$$
(I)

$$CH_3.CH{=}CH{-}C{\equiv}C{-}C{\equiv}C{-}(CH_2)_2COOH.$$
(II)

The acid (I) was readily obtained pure by hydrolysing the glycerides and crystallizing the free acids from acetone and from light petroleum at low temperatures. It forms colourless lath-like crystals up to 2 cm in length which melt slowly from 42·2–43 °C and which quickly become deep blue when exposed to light.

The structure assigned I is based on the following evidence. Catalytic hydrogenation effects the addition of 5 molecules of hydrogen and gives pure stearic acid. The chief products obtained from oxidation with neutral permanganate are valeric, oxalic, and azelaic acids. Ozonization, followed by reduction, yields valeraldehyde. The structure I is thus established; the presence of a strong absorption band at 955 $cm^{-1}$ in the infra-red spectrum shows that the ethylenic bond is of *trans* configuration, and weak, but significant, bands at 2185 and at 2215 $cm^{-1}$ provide additional evidence for the presence of two acetylenic bonds.

The ultraviolet absorption spectrum of this acid is drawn in Figure 1. Maxima occur at 214·5, 229, 240, 252·5, 266·5, and 282 mμ with extinction coefficients of ε 58500, 3500, 7000, 14900, 23300, and 17000. Christensen and Sörensen (1952) have described the ultraviolet spectrum of dec-*trans*-8-en-4,6-diynoic acid (II) which contains the same chromophore. The resemblance between the spectra is very close except that these authors did not explore the spectrum at shorter wavelengths and therefore did not observe the very intense band at 214·5 mμ. They report the weak band at 227·5 mμ for the isomeric *cis* acid, but omit it for the *trans* acid for which they report:

| | | | | |
|---|---|---|---|---|
| $\lambda_{max.}$ .. | 239·0 | 251·0 | 265·3 | 281·4 |
| $\varepsilon_{max.}$ .. | 10720 | 16600 | 22650 | 17780 |

Octadec-*trans*-13-en-9,11-diynoic acid (I) has been synthesized by Crombie and Jacklin (1957) although they could obtain it only as an uncrystallizable yellow oil and somewhat impure. It must have been principally our acid for

they reported five bands in the ultraviolet absorption spectrum with the following characteristics which agree remarkably well with ours.

| | | | | | | |
|---|---|---|---|---|---|---|
| $\lambda_{max.}$ | .. | 229 | 240 | 253 | 267 | 283 |
| $\varepsilon_{max.}$ | .. | 3000 | 6000 | 12500 | 20000 | 15500 |

Like Christensen and Sörensen they did not describe the pronounced absorption at 214·5 mμ although this seems to be typical of an enediyne chromophore (cf., for example, the observation of Bu'Lock, Jones, and Leeming (1955) concerning nemotinic acid).

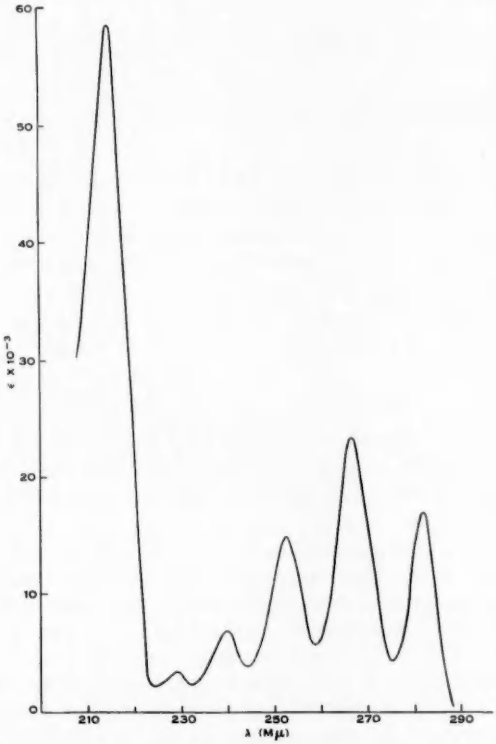


Fig. 1.—Ultraviolet absorption spectrum of I in hexane.

Preliminary investigations have been made of extracts of other parts of this plant. Essentially the same mixture of glycerides is present in both the bark and the wood of the roots. The octadecendiynoic acid (I) is present also in the wood of the trunk and of the main branches, but it appears to be absent from the foliage (branchlets) and from the fleshy highly coloured fruit stalk and it is not present in the seed oil.

Preliminary examinations have been made of other related plant materials. The species *Exocarpus stricta* R.Br. is a shrub found in Victoria. Only small quantities of this have been available. A sample of root when extracted with acetone gave an oil possessing the typical ultraviolet spectrum of an enediyne compound with extinction coefficients at the maxima corresponding to the presence of 53 per cent. of octadec-13-en-9,11-diynoic glycerides. The crystalline acid (I) was isolated from the oil after hydrolysis. The small seeds of this species when extracted with light petroleum gave a fat showing the typical ultraviolet absorption spectrum of ximenynic glycerides.

A sample of the fresh roots of *Ximenia americana* Linn. obtained from northern Queensland, when extracted with acetone gave an extract having an ultraviolet spectrum which closely resembles that of acid I. However, the extract proved more difficult to resolve than that from the roots of *E. cupressiformis* and a pure acid has not been isolated. This species of *Ximenia* is one of those from the fruits of which South African workers (Ligthelm and Schwartz 1950; Ligthelm, Schwartz, and von Holdt 1952) first isolated ximenynic acid and these preliminary experiments suggest root and seed fats may bear a similar chemical relationship to that now reported for an *Exocarpus* species.

A fuller investigation of these root and seed oils is contemplated. The remarkable fact that an acetylenic acid (octadecenynoic) can form the principal acid of the seed fat and an entirely different acetylenic acid (octadecenediynoic) the principal acid of the root fat, makes a fuller examination of the fats of species of the Santalales, particularly from parts other than the seeds, of considerable interest.

## II. Experimental

### (*a*) *The Seed Oil of* Exocarpus cupressiformis

The fruit ripens mainly in November. Material was collected chiefly from the Warrandyte and Mt. Evelyn districts, Victoria. Ultraviolet examination of light petroleum extracts of the fleshy stem showed the absence of any polyacetylenic material and this portion of the fruit was rejected. The small ovoid nuts (3·5 by 4·5 mm) had an average weight of 0·048 g and contained 23% of moisture. The dried nuts of one sample contained 43·5% of kernel and this kernel 58·4% of oil. After extraction of the oil the residual kernel meal contained 7·9% N. The seed fat was conveniently extracted directly from the nuts by crushing and subsequent extraction with hot hexane under nitrogen. After 2 hr, the material was crushed again and extracted for a further 2 hr. The nuts yielded 25·3% of oil. Later samples gave up to 31% oil. This oil was viscous, resembling crude oil from the sweet quandong (*Santalum acuminatum*). When mixed with acetone, it left 2·3–2·6% of a "rubber" undissolved. Removal of the acetone under reduced pressure in nitrogen then left a mobile oil of the following properties: $n_D^{20}$ 1·4870; $\Delta_4^{20}$ 0·9610, very slight optical rotation, acid value 1·2, saponification value 188, iodine value (1 hr Wijs) 106. A microdetermination of glycerol yielded 9·3%. The non-saponifiable matter was 2·4%. The infra-red spectrum showed a single acetylenic peak at 2210 $cm^{-1}$. In the ultraviolet spectrum the maximum at 228·5 mμ had an intensity corresponding to 62·3% of ximenynic acid as glycerides. In other samples the content of these glycerides ranged from 61 to 64%.

The oil was saponified by shaking it for 1 hr at room temperature with a solution of 0·75 part of potassium hydroxide in 10 parts of ethanol. After standing 12 hr excess of water was added, the unsaponifiable matter was extracted with light petroleum, and the fatty acids then recovered by acidification and extraction in light petroleum. The yield of fatty acids was 93·5%.

*(b) Isolation of the Ximenynic Acid*

The total fatty acids (11·1 g) were separated into six fractions by precipitation from methanol solution with urea. The first three fractions contained progressively decreasing proportions of saturated acids and increasing proportions of ximenynic acid. Fractions four and five were almost pure ximenynic acid and final purification was by crystallization twice from light petroleum (b.p. <40 °C) and once from acetone. The acid formed large colourless plates, m.p. 37·5–38·5 °C not depressed on admixture with an authentic synthetic sample.

The ultraviolet spectrum of the acid showed a maximum at 228 mμ (ε, 16,400), and an inflexion at 240 mμ (ε, 12,000).

The *p*-phenylphenacyl ester prepared by the standard procedure, had m.p. 63–63·5 °C, either alone, or admixed with an authentic sample.

*(c) The Root Oil of* Exocarpus cupressiformis

All extractions of this oil were made on roots obtained from the same tree, in the Panton Hill district north of Melbourne. Material obtained at different times over a period of several months did not differ noticeably in oil content. In a typical extraction, the fresh roots (3·2 kg) were broken up in a disintegrator mill, were covered with acetone (6 l.), and then stirred gently for 1 hr. After filtration and pressing to free of solvent, the residues were extracted once more in like manner. A third extraction yielded a negligible amount of oil. The acetone was removed from the extract in a climbing film evaporator at room temperatures under reduced pressure and the residual aqueous liquors, on which floated a greenish yellow oil, were extracted three times with light petroleum (b.p. 40–60 °C), the extracts washed, dried over sodium sulphate, and the solvent removed under reduced pressure in nitrogen. The resulting oil deposited black tarry material while standing for 24 hr at 0 °C and when filtered from this was a clear deep yellow colour. Yield 43 g (1·34%). Absorption at 266·5 mμ showed that the oil contained 58·6% of the octadecenediynoic acid (I) as glycerides. In other experiments the bark and woody portions of the roots were extracted separately. The former contained between 60 and 70% moisture, the latter 30–40%. Determination of oil content gave variable results, but the contents in the two portions were of the same order and little purpose was served in extracting the two classes of plant material separately.

The clear yellow oil was shown to consist essentially of glycerides. The saponification number of 188 corresponded to an expected yield of glycerol of 10·3%. Microestimations of glycerol gave the lower values of 8·6, 8·9%. The oil proved difficult to saponify with cold alkali and hydrolysis was achieved by refluxing with 0·5N alcoholic potassium hydroxide for 1 hr under nitrogen. Unsaponifiable matter was extracted from a 1 : 2 ethanol–water solution, first with light petroleum and subsequently with diethyl ether. The total amount of unsaponifiable material extracted was 6%. The fatty acids were then liberated by acidification with hydrochloric acid and extracted in diethyl ether; yield 86%. The presence of glycerol in the residual aqueous alcoholic liquors was established by neutralizing with alkali, concentrating to remove the alcohol, and subsequent conversion of the glycerol in solution into its insoluble tribenzoate, m.p. 72–73 °C, either alone or mixed with an authentic specimen.

The crude total acids from this oil were crystalline. Repeated crystallization from acetone and from light petroleum at low temperatures gave the pure octadec-*trans*-13-en-9,11-diynoic acid as colourless lath-like crystals of more than 2 cm length, m.p. 42·2–43 °C (Found: C, 78·8; H, 9·4; O, 12·3%; equiv. wt., 272·3. Calc. for $C_{18}H_{26}O_2$: C, 78·8; H, 9·6; O, 11·7%; equiv. wt., 274·3). On exposure to light the crystals quickly developed a blue colour due to the formation of an insoluble pigment. This pigment could be readily separated from unchanged acid by dissolving the latter in acetone, but on contact with this solvent the blue pigment at once became deep red in colour. The ultraviolet absorption spectrum of the pure acid is shown in Figure 1. The infra-red spectrum of a "Nujol mull" showed weak bands due to C≡C stretching at 2185 and 2215 $cm^{-1}$; a weak band, C=C stretching, at 1625 $cm^{-1}$, and the strong band for the out-of-plane CH deformation of a *trans*-ethylene bond at 955 $cm^{-1}$. The p-*bromophenacyl ester* prepared in the usual manner and crystallized from alcohol, formed colourless crystals, m.p. 57–58 °C (Found: C, 66·2; H, 6·8; O, 10·4; Br, 17·1%. Calc. for $C_{26}H_{31}O_3Br$: C, 66·2; H, 6·6; O, 10·2; Br, 16·9%).

When hydrogenated in ethyl acetate with platinum oxide as catalyst the acid (55 mg) absorbed 5·5 mol. equiv. (28·3 ml) of hydrogen and was converted to stearic acid of m.p. 68–69·5 °C either alone or mixed with an authentic specimen.

The acid (2 g), in solution in acetone (40 ml) and water (4 ml) at 3 °C, was oxidized by the gradual addition of potassium permanganate (9 g) and sodium bicarbonate (3 g) as a suspension in acetone (200 ml). After standing 15 hr, the acetone was removed and the residual oxide of manganese extracted with hot water. After evaporation of the colourless aqueous extracts, they were acidified and extracted repeatedly with diethyl ether. The residual aqueous liquors, after freeing of ether and neutralizing with aqueous ammonia, gave a white precipitate with calcium chloride, insoluble in dilute acetic acid. This precipitate (0·53 g) was identified as calcium oxalate by the colour test with 2,7-dihydroxynaphthalene after reduction with magnesium, and by the colour test with diphenylamine and phosphoric acid.

The ether extracts above were freed of ether and steam distilled. Light petroleum extraction of the distillate yielded a liquid acid with the typical odour of valeric acid and containing as its principal fraction a liquid of b.p. 174–180 °C. This fraction was converted to its *p*-bromophenacyl ester, m.p. 63–64 °C, from alcohol. Admixture with authentic *p*-bromophenacyl valerate did not depress the melting point.

The aqueous residue from the steam distillate contained a crystalline acid melting at first at 95–104 °C and displaying an infra-red spectrum which showed the weak absorption band of an acetylenic bond at 2250 $cm^{-1}$. This was attributed to the presence of an acetylenic dicarboxylic acid in the main product (azelaic acid), as a result of incomplete oxidation. Repeated recrystallization from water raised the m.p. to 103–106 °C (yield 0·44 g) and this material depressed the melting point of pimelic acid, but not that of azelaic acid. Its di-*p*-bromophenacyl ester melted at 132·5–133 °C and was undepressed when mixed with an authentic sample of di-*p*-bromophenacyl azelate (Found: Br, 27·6%. Calc. for $C_{25}H_{26}O_6Br_2$: Br, 27·5%).

The octadec-*trans*-13-en-9,11-diynoic acid (2 g) was also oxidized by ozonization in ethyl acetate (30 ml) by passing ozonized oxygen (5 molecular equivalents of ozone) for 2 hr and subsequently hydrogenating the products using palladized calcium carbonate as catalyst. The products were freed of solvent and steam distilled. The distillate possessed the characteristic odour of valeraldehyde. Extraction with light petroleum gave valeraldehyde as an oil; its 2,4-dinitrophenylhydrazone melted at 104–105 °C, either alone or mixed with an authentic sample.

The aqueous residues from steam distillation when freed of tar and cooled deposited crystals of an acidic material, m.p. 93–103 °C. Its infra-red spectrum also showed a weak band typical of an acetylenic bond, but after further oxidation of the acid with alkaline permanganate this band was eliminated and the acid from this further oxidation was shown to be azelaic acid, m.p. 106 °C.

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# STUDIES IN THE CHEMISTRY OF PHENOTHIAZINE

## IV. THE PREPARATION OF 2,2′-DINITRODIPHENYLSULPHIDES AND THEIR CONVERSION TO PHENOTHIAZINES

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*Summary*

The preparation of phenothiazine, and of some substituted phenothiazines from the 2,2′-dinitrodiphenylsulphides, is described.

### I. Introduction

Certain substituted phenothiazines can only be made conveniently and unambiguously, by means of the Smiles rearrangement of the appropriate diphenylsulphides. The sulphides are prepared from the *o*-aminobenzenethiols and the *o*-halonitrobenzenes. Substituted *o*-aminobenzenethiols are difficult to prepare, and their syntheses involve lengthy processes affording only small yields. In this Laboratory it has been shown that, in certain cases, phenothiazines can be prepared from 2,2′-dinitrodiphenylsulphides by a selective reduction of a nitro group in the presence of alkali. Phenothiazines can also be obtained, in low yields, by the direct action of sodium sulphide on *o*-halonitrobenzenes, in a high boiling solvent. A Smiles rearrangement and ring closure takes place in these methods without the necessity of isolating and acetylating the amine intermediate.

### II. Diphenylsulphides

The dinitrodiphenylsulphides were prepared either by the action of sodium sulphide on the *o*-halonitrobenzenes, or by the condensation of the sodium salt of the *o*-nitrobenzenethiol with the *o*-halonitrobenzenes. No sulphide was obtained when 2,4-dichloronitrobenzene or 4-chloro-3-nitrotoluene (Hodgson and Ward 1948) reacted with sodium sulphide. 2,3-Dichloronitrobenzene gave negligible quantities of a sulphide which contained some amino compound. However, when 2,3-dichloronitrobenzene reacted with sodium sulphide in diethylene glycol instead of ethanol as the solvent, the product obtained appeared to be a sulphide which contained only one chlorine atom.

### III. Phenothiazines

Phenothiazine was formed when 2,2′-dinitrodiphenylsulphide was treated with hydrazine hydrate in the presence of sodium hydroxide. Similarly, 4,4′-dichloro-2,2′-dinitrodiphenylsulphide gave 2,7-dichlorophenothiazine (Farrington and Warburton 1955), which indicated that a Smiles rearrangement

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had taken place. Sodium acetate can replace the sodium hydroxide and ethanol or diethylene glycol is a suitable solvent. It was also found that 2,7-dichlorophenothiazine could be prepared by the action of sodium sulphide on the dinitrodiphenylsulphide, if diethylene glycol was used as the reaction solvent. The best yields of phenothiazines were obtained from symmetrically substituted sulphides.

The direct action of sodium sulphide on some *o*-halonitrobenzenes in diethylene glycol gave low yields of phenothiazines. This method was useful for preparing 2,7-dimethylphenothiazine which could not be prepared by other means. Phenothiazine itself could not be obtained by this method although 2,7-dichlorophenothiazine and 2,7-dibromophenothiazine were prepared.

## IV. Experimental

Analyses are by Dr. K. W. Zimmermann, C.S.I.R.O. Microanalytical Laboratory.

(*a*) *2,2′-Dinitrodiphenylsulphide.*—A solution of hydrated sodium sulphide (15 g) in water (10 ml) was added to a refluxing solution of *o*-chloronitrobenzene (20 g) in 95% ethanol (50 ml). After refluxing for 6 hr the unreacted *o*-chloronitrobenzene (13·8 g) was recovered by steam distillation. The residue was recrystallized from ethyl acetate–ethanol to give yellow needles (2·7 g, 50%), m.p. 122 °C; Hodgson and Ward (1948) report m.p. 122 °C (Found: C, 52·5; H, 3·1; O, 23·1; N, 9·8%. Calc. for $C_{12}H_8O_4N_2S$: C, 52·2; H, 2·9; O, 23·2; N, 10·1%).

(*b*) *Phenothiazine.*—Sodium hydroxide (0·8 g) and 2,2′-dinitrodiphenylsulphide (2 g) were dissolved with heating in diethylene glycol (15 ml). To the refluxing solution was added hydrazine hydrate (0·4 g), and 30 min later more hydrazine hydrate (0·8 g) was added to the mixture. After a further hour, the reaction mixture was poured into a saturated salt solution and left in the refrigerator for some hours. The fawn coloured precipitate, after purification by passage of its benzene solution through an alumina column and recrystallization from ethanol (charcoal) gave yellow plates (0·5 g, 35%), m.p. and mixed m.p. with phenothiazine 185 °C (Found: C, 72·3; H, 4·9; N, 7·1%. Calc. for $C_{12}H_9NS$: C, 72·3; H, 4·6; N, 7·0%).

(*c*) *4-Chloro-2,2′-dinitrodiphenylsulphide.*—The reaction of *o*-nitrobenzenethiol (2·3 g) with sodium hydroxide and 2,5-dichloronitrobenzene (3·1 g) gave yellow needles (2·2 g, 48%), m.p. 139 °C, from acetic acid (Found: C, 46·6; H, 2·5; N, 8·6; Cl, 11·1%. Calc. for $C_{12}H_7O_4N_2SCl$: C, 46·4; H, 2·3; N, 9·0; Cl, 11·4%). The action of a large excess of hydrazine hydrate in the presence of alkali on the above compound gave a low yield of straw coloured needles, m.p. 196 °C, from benzene–light petroleum. Qualitative tests indicated the presence of a phenothiazine (Found: N, 5·7%. Calc. for $C_{12}H_8NSCl$: N, 6·0%).

(*d*) *6-Chloro-2,2′-dinitrodiphenylsulphide.*—The reaction of *o*-nitrobenzenethiol (3·0 g) with sodium hydroxide and 2,3-dichloronitrobenzene (4·0 g) gave lemon needles (4·3 g, 72%), m.p. 172 °C, from ethanol–acetone (Found: C, 46·4; H, 2·3; N, 8·6%. Calc. for $C_{12}H_7O_4N_2SCl$: C, 46·4; H, 2·3; N, 9·0%). When the compound was treated as in (*b*) the presence of a phenothiazine was confirmed by qualitative tests, but the yield was too low for isolation and identification.

(*e*) *4,4′-Dichloro-2,2′-dinitrodiphenylsulphide.*—When 2,5-dichloronitrobenzene (24 g) was treated as in (*a*), unreacted starting material (7·7 g) was recovered by steam distillation. The residue gave yellow needles (8·9 g, 61%), m.p. 149–150 °C, from acetic acid; Beilstein and Kurbatow (1879) report m.p. 149–150 °C (Found: C, 42·1; H, 2·1; Cl, 20·7%. Calc. for $C_{12}H_6O_4N_2SCl_2$: C, 41·8; H, 1·7; Cl, 20·6%).

(*f*) *2,7-Dichlorophenothiazine.*—(i) The reaction described in (*b*) was carried out on 4,4′-dichloro-2,2′-dinitrodiphenylsulphide (2 g) to give colourless plates (0·8 g, 52%) from benzene–light petroleum, m.p. 216 °C, undepressed by authentic 2,7-dichlorophenothiazine for which Farrington and Warburton (1955) report m.p. 216–217 °C (Found: C, 54·0; H, 2·8; N, 5·1%. Calc. for $C_{12}H_7NSCl_2$: C, 53·7; H, 2·6; N, 5·2%).

(ii) When 4,4′-dichloro-2,2′-dinitrodiphenylsulphide (3 g) and crystalline sodium sulphide (12 g) were reacted in diethylene glycol for 2 hr, a fawn coloured precipitate was collected after pouring the reaction mixture into water. After chromatography and recrystallization from benzene–light petroleum the product (0·6 g, 24%) was obtained as colourless plates, m.p. and mixed m.p. 216 °C.

(iii) A solution of anhydrous sodium sulphide (12·5 g) and 2,5-dichloronitrobenzene (10 g) in diethylene glycol (20 ml) was refluxed for 4 hr and then poured into water. The product (0·25 g), purified as above, had m.p. and mixed m.p. 216 °C.

(*g*) *4,5′-Dichloro-2,2′-dinitrodiphenylsulphide.*—The reaction of 4-chloro-2-nitrobenzenethiol (1·9 g) and sodium hydroxide with 2,4-dichloronitrobenzene (2·2 g) gave yellow clustered needles (1·2 g, 35%) from acetic acid, m.p. 184 °C (Found: C, 42·1; H, 1·9; N, 8·0%. Calc. for $C_{12}H_6O_4N_2SCl_2$: C, 41·8; H, 1·7; N, 8·1%). When this compound was treated as in (*b*), the presence of a phenothiazine was detected by qualitative tests, but the yield was so low that the compound was not isolated.

(*h*) *4,6′-Dichloro-2,2′-dinitrodiphenylsulphide.*—The reaction of 4-chloro-2-nitrobenzenethiol (8 g) and sodium hydroxide with 2,3-dichloronitrobenzene (9·3 g) gave yellow needles (7·1 g, 49%) from acetic acid, m.p. 132 °C (Found: C, 41·9; H, 1·9; N, 8·0%. Calc. for $C_{12}H_6O_4N_2SCl_2$: C, 41·8; H, 1·7; N, 8·1%). When the compound was treated as in (*b*), qualitative tests indicated the presence of a phenothiazine in very poor yield.

(*i*) *4,4′-Dibromo-2,2′-dinitrodiphenylsulphide.*—When 2,5-dibromonitrobenzene was treated as in (*a*), yellow needles formed in the reaction mixture almost immediately. After refluxing for 3 hr the compound was collected and recrystallized from acetone-ethanol to give yellow needles (59%), m.p. 165 °C; Blanksma (1901) gives m.p. 165 °C (Found: C, 33·2; H, 1·7; N, 6·3%. Calc. for $C_{12}H_6O_4N_2SBr_2$: C, 33·2; H, 1·4; N, 6·5%).

(*j*) *2,7-Dibromophenothiazine.*—(i) The action of hydrazine hydrate and alkali on 4,4′-dibromo-2,2′-dinitrodiphenylsulphide as in (*b*) did not give a phenothiazine. However, treatment of the compound (2 g) with a large excess of hydrazine hydrate (6 ml), gave a product (10%) which crystallized from benzene–light petroleum as fawn needles, m.p. 213·5 °C (decomp.) (Found: C, 40·6; H, 2·1; N, 3·9%. Calc. for $C_{12}H_7NSBr_2$: C, 40·3; H, 2·0; N, 3·9%).

(ii) 2,5-Dibromonitrobenzene (10 g) when treated as in (*f*) (iii) gave a poor yield of fawn needles, m.p. and mixed m.p. 213 °C (decomp.).

(*k*) *2,2′, 4,4′-Tetranitrodiphenylsulphide.*—When 1-chloro-2,4-dinitrobenzene was treated as in (*a*), a vigorous reaction took place. The product was obtained in high yield (84%) as fawn coloured plates, m.p. 196 °C, from acetic acid (Found: C, 39·3; H, 1·9; N, 15·0%. Calc. for $C_{12}H_6O_8N_4S$: C, 39·3; H, 1·6; N, 15·3%). No phenothiazine was obtained when the compound was treated with hydrazine as in (*b*).

(*l*) *2,7-Dimethylphenothiazine.*—The reaction of 4-chloro-3-nitrotoluene (10 g) with sodium sulphide as in (*f*) (iii) gave cream plates (0·17 g), m.p. 213 °C, from benzene–light petroleum (Found: C, 74·3; H, 5·7; N, 5·8; S, 13·9%. Calc. for $C_{14}H_{13}NS$: C, 74·0; H, 5·7; N, 6·2; S, 14·1%).

(*m*) *Reaction of 2,3-Dichloronitrobenzene with Sodium Sulphide in Diethylene Glycol.*—When 2,3-dichloronitrobenzene (10 g) was treated with a large excess of hydrated sodium sulphide (12 g) as in (*f*) (iii), the product (0·7 g) was obtained as dark purple needles, m.p. 202 °C, after recrystallization from aqueous acetic acid (Found: C, 52·0; H, 2·5; N, 9·5; O, 11·7; S, 11·3; Cl, 13·3%. Calc. for $C_{12}H_7O_5N_2SCl$: C, 51·7; H, 2·5; N, 10·1; O, 11·5; S, 11·5; Cl, 12·8%). The molecular weight could not be determined by the Rast method because of the dark colour of the camphor solution.

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# THE INFLUENCE OF IONIZATION ON THE ULTRAVIOLET SPECTRA OF CHLORINATED PHENOXYACETIC ACIDS AND RELATED PHOSPHORUS ANALOGUES

By J. N. PHILLIPS*

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*Summary*

Ionization has been shown to influence the ultraviolet spectra of compounds of the general type Ph—O—$CH_2$—X, where X is an ionizable group. It is suggested that this influence arises from an inductive effect of the charge on the electronic interaction between the aromatic ring and the phenolic oxygen atom. The ionization effect can be used to determine the dissociation constants of such compounds spectroscopically.

## I. INTRODUCTION

During an investigation of the ultraviolet absorption spectra of a number of chlorinated phenoxyacetic acids it was observed that ionization of the carboxyl group invariably tended to displace the spectra towards the visible region.

Although it is a common phenomenon for ionization to modify the spectroscopic behaviour of aromatic acids and bases it is generally associated with those compounds whose acidic or basic centres are directly involved in the $\pi$-electron mobility in the molecule. Such cannot be the case here, however, since the ionizable acidic group is separated from the aromatic region by means of a methylene group. It suggests that the ionization effect operates by an inductive mechanism.

This has been confirmed by later experiments which have indicated that the ionization effect is independent of the nature of the ionizing group, but dependent on the sign and magnitude of the charge on the ionic species and on the distance of separation between the charge and the phenolic oxygen atom.

## II. EXPERIMENTAL

The ultraviolet absorption measurements were carried out using 1 cm cells in a Beckmann spectrophotometer at 20 °C, the concentration of the solution being usually $5 \times 10^{-4}$M. The absorption results are expressed in terms of the molar extinction coefficient ($\varepsilon$).

The spectrum of the ionic form was generally determined in a 0·01M phosphate buffer (pH 5–8) and that of the undissociated acid in hydrochloric acid solution. 0·1N HCl was sufficiently acidic to effectively repress ionization in the phenoxyacetic acid series but with the phosphorus analogues 7·5N HCl was used. Even in this highly acidic medium corrections had to be applied

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TABLE 1

$\varepsilon_{max.}$ $\lambda_{max.}$ VALUES FOR THE B ABSORPTION BANDS OF THE IONIZED AND UNDISSOCIATED SPECIES OF CHLORINATED PHENOXYACETIC ACIDS AND RELATED PHOSPHORUS ANALOGUES

| Compound | Positions of Chlorine Substitution | Undissociated Molecule | | | | Mono-Ion | | | | Di-Ion | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $\lambda_1$ (Å) | $\varepsilon_1 \times 10^{-2}$ | $\lambda_2$ (Å) | $\varepsilon_2 \times 10^{-2}$ | $\lambda_1$ (Å) | $\varepsilon_1 \times 10^{-2}$ | $\lambda_2$ (Å) | $\varepsilon_2$ $10^{-2}$ | $\lambda_1$ (Å) | $\varepsilon_1 \times 10^{-2}$ | $\lambda_2$ (Å) | $\varepsilon_2 \times 10^{-2}$ |
| Phenoxyacetic acid | None | 2680 | 13·0 | 2745 | 10·7 | 2695 | 14·2 | 2755 | 11·6 | | | | |
| | 2 | 2720 | 16·0 | 2790 | 14·2 | 2735 | 18·4 | 2805 | 16·2 | | | | |
| | 3 | 2720 | 15·2 | 2795 | 13·7 | 2735 | 16·3 | 2805 | 14·6 | | | | |
| | 4 | 2780 | 12·9 | 2840* | 10·3 | 2790 | 13·5 | 2855* | 10·9 | | | | |
| | 2,4 | 2820 | 18·2 | 2890* | 15·7 | 2835 | 20·7 | 2905* | 18·4 | | | | |
| | 2,5 | 2785 | 20·8 | 2860 | 19·3 | 2795 | 23·5 | 2870 | 21·9 | | | | |
| | 2,6 | 2705 | 2·9 | 2765 | 2·5 | 2710 | 3·7 | 2780 | 3·4 | | | | |
| | 3,4 | 2815 | 15·5 | 2890 | 14·0 | 2825 | 16·9 | 2895 | 15·2 | | | | |
| | 2,3,4 | 2845 | 15·3 | 2925 | 15·7 | 2855 | 17·2 | 2935 | 17·8 | | | | |
| | 2,3,6 | 2760 | 3·2 | 2845 | 2·7 | 2765 | 3·9 | 2850 | 3·4 | | | | |
| | 2,4,5 | 2875 | 22·6 | 2950 | 21·2 | 2885 | 26·0 | 2965 | 24·5 | | | | |
| | 2,4,6 | 2785 | 6·6 | 2865 | 6·6 | 2795 | 7·0 | 2870 | 7·1 | | | | |
| Phenoxymethyl-phosphonic acid | None | 2685 | 12·8 | 2745 | 10·8 | 2690 | 13·7 | 2755 | 11·4 | 2705 | 17·2 | 2765 | 14·0 |
| | 2 | 2725 | 15·6 | 2795 | 13·6 | 2735 | 17·2 | 2800 | 15·2 | 2745 | 20·3 | 2815 | 17·9 |
| | 2,4 | 2820 | 17·3 | 2895* | 14·8 | 2830 | 19·2 | 2905 | 16·7 | 2845 | 21·3 | 2915 | 19·0 |
| | 2,6 | 2705 | 3·1 | 2775* | 2·6 | 2705 | 3·6 | 2775 | 3·1 | 2710 | 4·6 | 2780 | 4·0 |
| | 2,4,5 | 2870 | 21·2 | 2950 | 20·0 | 2880 | 22·8 | 2960 | 21·5 | 2890 | 25·0 | 2970 | 24·0 |

* Shoulder in the $\varepsilon$–$\lambda$ curve.

TABLE 1 (*Continued*)

| Compound | Positions of Chlorine Substitution | Undissociated Molecule | | | | Mono-Ion | | | | Di-Ion | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $\lambda_1$ (Å) | $\varepsilon_1 \times 10^{-2}$ | $\lambda_2$ (Å) | $\varepsilon_2 \times 10^{-2}$ | $\lambda_1$ (Å) | $\varepsilon_1 \times 10^{-2}$ | $\lambda_2$ (Å) | $\varepsilon_2 \times 10^{-2}$ | $\lambda_1$ (Å) | $\varepsilon_1 \times 10^{-2}$ | $\lambda_2$ (Å) | $\varepsilon_2 \times 10^{-2}$ |
| Ethyl(phenoxymethyl) phosphonate | None | | | | | | | | | | | | |
| | 2 | 2725 | 15·6 | 2795 | 13·6 | 2735 | 17·2 | 2800 | 15·1 | | | | |
| | 4 | 2775 | 13·0 | 2840* | 10·2 | 2785 | 13·6 | 2855* | 10·6 | | | | |
| | 2,4 | 2820 | 17·2 | 2895* | 14·8 | 2830 | 18·8 | 2905* | 16·4 | | | | |
| | 2,6 | 2700 | 3·2 | 2765* | 2·7 | 2705 | 3·7 | 2770* | 23·1 | | | | |
| | 2,4,5 | 2870 | 22·5 | 2950 | 21·2 | 2880 | 24·2 | 2960 | 22·8 | | | | |
| | 2,4,6 | 2790 | 5·0 | 2870 | 5·0 | 2790 | 6·3 | 2870 | 6·4 | | | | |
| Phenoxymethylphosphinic acid | None | | | | | | | | | | | | |
| | 4 | 2780 | 13·4 | 2840* | 10·7 | 2790 | 13·7 | 2850* | 11·2 | | | | |
| | 2,4 | 2825 | 17·0 | 2890* | 14·6 | 2835 | 18·5 | 2895* | 16·4 | | | | |
| | 2,4,5 | 2875 | 22·5 | 2955 | 21·3 | 2885 | 23·4 | 2960 | 21·3 | | | | |

* Shoulder in the $\varepsilon$–$\lambda$ curve.

to the observed values based on the $pK_a$ of the acid and the H-acidity function for 7·5N HCl (Phillips 1958).

The phosphorus analogues were of analytical purity (Maguire and Shaw 1953).* The phenoxyacetic acids were commercial samples whose purity judged from potentiometric titration evidence was >98 per cent.

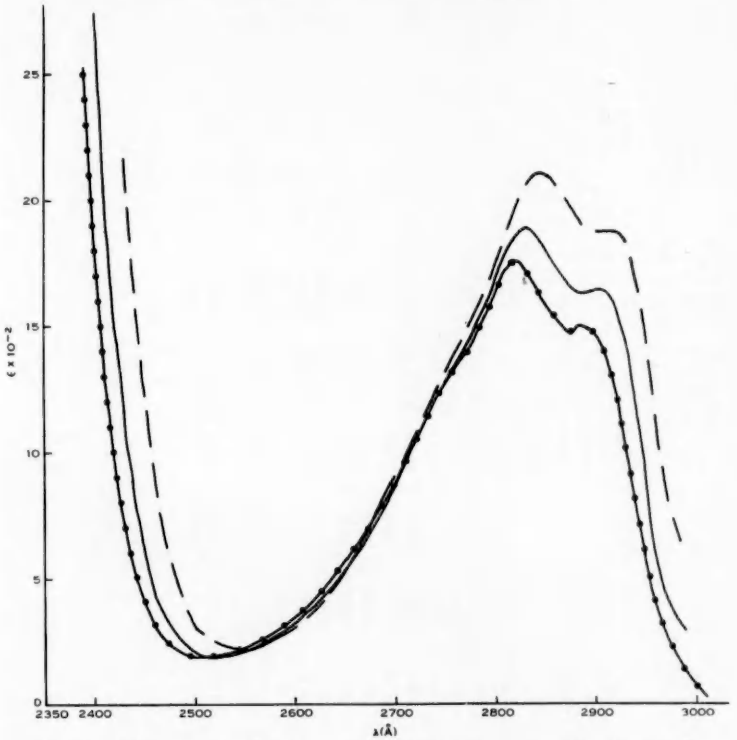


Fig. 1.—The effect of ionization on the ultraviolet spectra of 2,4-dichlorophenoxymethylphosphonic acid.
–·—·– Undissociated acid (6N HCl).
——— Mono-ion (pH=3·3).
— — Di-ion (pH=8·0).

Compounds of the phenolic type generally show a high intensity ($\varepsilon \sim 10^4$) absorption band, termed a K band, in the ultraviolet region 2000–2400 Å together with one or two bands, termed B bands, of moderate intensity ($\varepsilon \sim 10^3$) in the region 2600–3000 Å (Burawoy and Chamberlain 1952). This investigation has been confined to a study of the B bands, the data recorded in Table 1 referring to the two B band absorption maxima.

* See Section III present paper.

The B band absorption maxima of the compounds investigated are determined primarily by the number and position of the chloro groups substituted in the phenyl ring, the nature of the acidic group having little influence except insofar as it be ionized or not. As will be observed from Table 1 the substitution of a chloro group generally leads to a bathochromic shift in the absorption maximum and an enhancement of its intensity.

2,6-Substituted compounds behave anomalously in that their B band absorption maxima occur at lower wavelengths and show a much reduced intensity when compared with their isomers. This has been attributed by various workers (Burawoy and Chamberlain 1952 ; Wiles 1956) to the substituent groups hindering free rotation about the phenolic oxygen with a consequent reduction in conjugation between the oxygen and the aromatic ring.

TABLE 2

INFLUENCE OF CHARGE TYPE ON THE $\lambda_1$ AND $\varepsilon_1$ VALUES OF A SERIES OF 2,4-DICHLOROPHENOXYMETHYL SUBSTITUTED COMPOUNDS

| Compound | Charge Type | $\lambda_1$ (Å) | $\varepsilon_1 \times 10^{-2}$ |
|---|---|---|---|
| $Cl_2—C_6H_3—OCH_2—N^+Et_3$* .. | + (Cation) | 2795 | 12·2 |
| $Cl_2—C_6H_3—OCH_2—CO_2H$ | O (Neutral) | 2820 | 18·2 |
| $Cl_2—C_6H_3—OCH_2—PO_3H_2$ | | 2820 | 17·3 |
| $Cl_2—C_6H_3—OCH_2—PO_2H_2$ | | 2825 | 17·0 |
| $Cl_2—C_6H_3—OCH_2—PO_3EtH$ | | 2820 | 17·2 |
| $Cl_2—C_6H_3—OCH_2—CO_2^-$ | — (Anion) | 2835 | 20·2 |
| $Cl_2—C_6H_3—OCH_2—PO_3H^-$ | | 2830 | 19·2 |
| $Cl_2—C_6H_3—OCH_2—PO_2H^-$ | | 2830 | 18·0 |
| $Cl_2—C_6H_3—OCH_2—PO_3Et^-$ | | 2830 | 18·8 |
| $Cl_2—C_6H_3—OCH_2—PO_3^=$ .. .. | = (Di-anion) | 2845 | 20·5 |

* Prepared by the method described by Barber and Green (1954).

The influence of ionization on the ultraviolet spectra of these acids is to displace the ε-λ curve towards the visible. The B band maxima in addition to undergoing a bathochromic shift of 5–15 Å show an increase of ~10 per cent. in intensity. The general effect is illustrated in Figure 1 for 2,4-dichlorophenoxymethylphosphonic acid and its mono- and di-anion.

It is of interest to note that Bandurski (1947) reported no change in the ultraviolet spectrum of 2,4-dichlorophenoxyacetic acid between a pH of 2·2 and 8·0, although its pK lies at 2·8 (Phillips 1958). Reference to Table 1 shows that Bandurski's reported wavelength maximum at 2835 Å and molar extinction coefficient of $2{\cdot}0 \times 10^3$ corresponds to that for the ionized species which would predominate in the pH region 2·8 to 8·0.

Table 2 illustrates the effect on the B band absorption maxima of a series of 2,4-dichlorophenoxymethyl substituted compounds obtained by varying the charge from monovalent positive to divalent negative. The spectral change observed is primarily a function of the nature and magnitude of the charge and could arise from the inductive effect of the charge on the electron distribution about the oxygen atom. It would be predicted that the more negatively charged the group the more the tendency to repel electrons from the oxygen atom, and

hence the greater the electronic interaction between the oxygen and the aromatic ring. This would lead to a shift in absorption maxima towards the visible and an enhancement of the intensity—as is observed.

Table 3 shows the effect of ionization on the position and intensity of one of the B band absorption maxima in the 2,4-dichlorophenoxyacetic, α-propionic, β-propionic, and γ-butyric acid series. The ionization effect diminishes with increasing distance of separation between the ionizing atom and the phenolic oxygen. This is consistent with the hypothesis that the shift is due to a field inductive effect.

TABLE 3

THE EFFECT OF IONIZATION ON THE POSITION AND INTENSITY OF THE $B_1$ BAND ABSORPTION MAXIMA IN A SERIES OF 2,4-DICHLOROPHENOXY SUBSTITUTED FATTY ACIDS

| Compound | $\lambda_1$ (Undissociated Molecule) (Å) | $\lambda_1$ (Ionized Species) (Å) | $\Delta\lambda_1$ (Å) | $\varepsilon \times 10^{-2}$ (Undissociated Molecule) | $\varepsilon \times 10^{-2}$ (Ionized Species) | $\Delta\varepsilon$ (%) |
|---|---|---|---|---|---|---|
| 2,4 - Dichlorophenoxyacetic acid .. .. | 2820 | 2835 | 15 | 18·2 | 20·7 | 14 |
| α-(2,4-Dichlorophenoxy)-propionic acid .. | 2825 | 2840 | 15 | 16·9 | 19·6 | 16 |
| β-(2,4-Dichlorophenoxy)-propionic acid .. | 2830 | 2835 | 5 | 17·9 | 18·8 | 5 |
| γ-(2,4-Dichlorophenoxy)-butyric acid .. .. | 2835 | 2835 | 0 | 16·8 | 17·3 | 3 |

The shift in the ultraviolet spectra induced by the ionization of the phenoxymethyl substituted acids can be used to determine their dissociation constants spectroscopically. It has been of particular use in the case of those compounds which were either too insoluble, for example, the poly-chlorinated phenoxyacetic acids, or too strongly acidic, for example, the phosphorus containing acids, for the more conventional potentiometric titration technique to be applied with accuracy. These results have been described elsewhere (Phillips 1958).

## III. Acknowledgments

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# SOME QUANTITATIVE INFRA-RED ABSORPTION STUDIES OF COALS, PYROLYSED COALS, AND THEIR ACETYL DERIVATIVES

By R. A. Durie* and S. Sternhell*

[*Manuscript received November* 6, 1958]

*Summary*

Quantitative studies have been made of the changes in the infra-red spectra of coals and chars that follow their acetylation. Those features in the spectra of the acetylated materials that are characteristic of the acetate groups were found to vary quantitatively with the chemically determined acetyl contents. Changes occurring in the hydroxyl absorption (at 3380 $cm^{-1}$) as a result of pyrolysis and acetylation tend to confirm that the 5 to 7 per cent. of oxygen which is not detected in the functional group analysis of Victorian brown coals is predominantly in the form of hydroxyl groups which resist acetylation and which appear to be stable on heating to about 450 °C.

## I. Introduction

Problems—such as particle size, control of mulling agent-sample ratios, and mull distribution and thickness—associated with the mulling technique used in most of the earlier infra-red studies on coal make quantitative studies very difficult. Brown (1955) has used the mulling technique for a comparison of the variation with carbon content of the relative absorption intensities of the aliphatic and aromatic CH groups in the spectra of several higher-rank bituminous coals.

Recently, the technique using potassium halide disks (Schiedt and Reinwein 1952; Stimson and O'Donnell 1952) has been used in a qualitative manner for coals (Bergmann *et al.* 1954; van Vucht, Rietveld, and van Krevelen 1955; Brooks, Durie, and Sternhell 1958*a*, 1958*b*). The control of sample concentration and disk thickness that is possible with this technique makes it potentially attractive for quantitative infra-red studies, although such factors as inadequate particle-size reduction and non-reproducible particle-size distribution, as well as the non-isotropic nature of the potassium halide sample mixture (Jones 1952; Jones and Sandorfy 1956) might be expected to cause difficulties. Despite these problems, several successful quantitative applications of the technique have been reported in the literature. Thus Kirkland (1955) has shown that Lambert's law is generally obeyed and has indicated the degree of reproducibility obtainable. Corbridge and Lowe (1955) report the analysis of condensed phosphate mixtures, Hayden (1955) that of hormones, and Wiberley, Sprague, and Campbell (1957) of polymers. O'Connor, DuPré, and McCall (1957) and Higgins (1957) illustrated the quantitative potentialities in the study of cellulose and cellulose derivatives.

The purpose of the present paper is to report the use of the potassium halide disk technique in a quantitative infra-red absorption investigation of acetylated coals and chars.

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## II. Experimental

Chars obtained by pyrolysis at temperatures up to 500 °C, from untreated Yallourn and Morwell brown coals, and from Yallourn brown coal after treatment with alkali at 185 °C (Brooks and Sternhell 1957), were acetylated and the acetyl

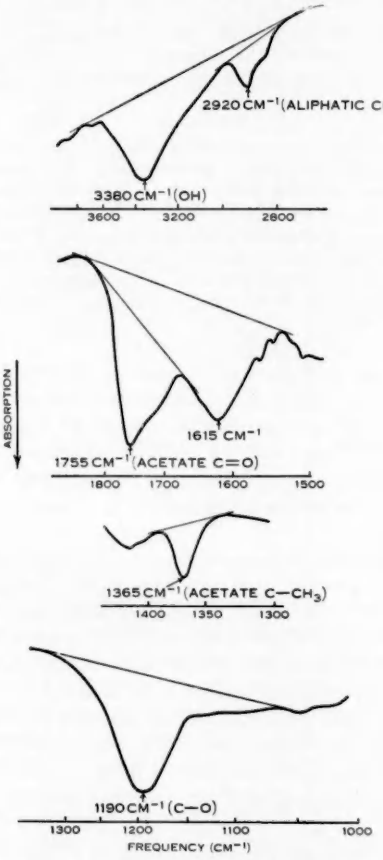


**Fig. 1.—Single-beam infra-red scans of an acetylated brown coal, showing base-line positions (0·25 per cent. in 200 mg KCl disk).**

groups determined chemically by the procedure of Brooks, Durie, and Sternhell (1958*b*). Five bituminous coals, ranging in carbon content from 84·0 to 89·9 per cent., were similarly acetylated.

Potassium chloride disks were prepared for infra-red studies according to the procedure described by Brooks, Durie, and Sternhell (1958*a*), except that the

bituminous-coal samples were ground for approximately 60 hr prior to mixing with potassium chloride. All spectra were recorded using a Perkin-Elmer Model 112 single-beam double-pass spectrometer fitted with a rock-salt prism. The normal string slit-drive schedule was used and each region was scanned three times, with a check of dark current position (100 per cent. absorption) at the start and finish of each scan.

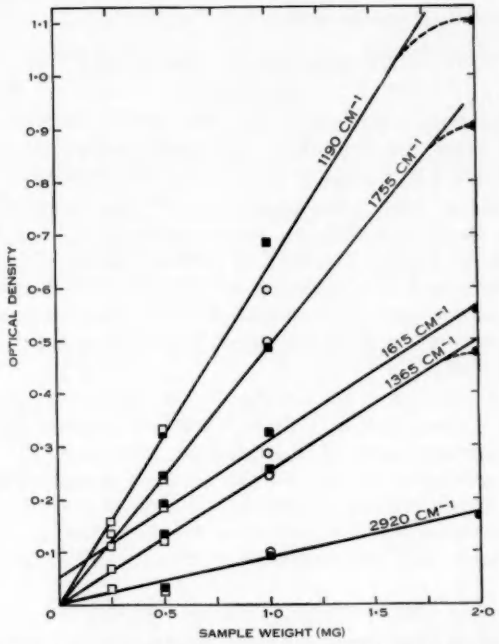


Fig. 2.—Yallourn brown coal; reproducibility of disk preparation, and linear relationship between optical density and sample weight.

□ 0·25 per cent. disks; ■ 0·5 per cent. disks; ○ 1·0 per cent. disk; ● 2·0 per cent. disk.

The base-line technique for measurement of the optical densities (Wright 1941) was employed throughout to minimize the effect of variable background levels caused by general scattering losses and, in the case of the higher-temperature chars, by the extension of the electronic absorption edge (McMichael, Kmetko, and Mrozowski 1954) into the infra-red region. The base-line positions used are shown in Figure 1.

To obtain information on the quantitative reproducibility of disk preparation and also on the sample " concentration " range, for which optical densities varied linearly with sample weight, a series of 100 and 200 mg potassium chloride disks were prepared containing 1·0, 0·5, and 0·25 per cent. of an acetylated brown

coal. Taking four key frequencies (1755, 1615, 1365, and 1190 $cm^{-1}$), the plot of base-line optical densities *versus* weight of sample in each disk (Fig. 2) shows that the optical densities,* with due allowance for the experimental scatter, vary linearly with sample weight in disks containing from 0·25 to more than 1·0 mg of coal. Some departure from linearity is indicated for disks containing 2 mg of coal. All the optical-density *versus* sample-weight lines pass through the origin except the 1615 $cm^{-1}$ line, which intersects the axis of ordinates at approximately +0·05 optical density unit.

## III. Results and Discussion

### (*a*) *General*

The standard optical densities at 3380, 2920, 1755, 1615, 1365, and 1190 $cm^{-1}$, together with relevant chemical data, are given in Table 1 for all the acetylated samples and for the parent coals or chars (except the Morwell chars).

The features at 1755, 1365, and 1190 $cm^{-1}$ characteristic of the acetate group (Brooks, Durie, and Sternhell 1958*a*) appeared in the spectra of all the acetylated coals and chars. The band at 1755 $cm^{-1}$ is characteristic of phenolic acetates and the persistent appearance of a distinct doublet at 1015–1040 $cm^{-1}$ and of a band at 890 $cm^{-1}$ in the spectra of all the acetylated samples indicates that such features are also characteristic of the acetate group. All these bands disappear on hydrolysis.

The optical densities of the acetate $C-CH_3$ absorption at 1365 $cm^{-1}$ and the acetate C—O absorption at 1190 $cm^{-1}$ are each proportional to the optical density of the acetate carbonyl absorption at 1755 $cm^{-1}$ (Fig. 3). Thus the environmental differences that may exist in the hydroxyl groups of different coals and chars are not such as to influence the infra-red absorption of the acetate group in their acetyl derivatives, and any of the above absorption bands may be used for correlation with the chemically-determined acetyl contents.

### (*b*) *Correlation of Acetate Group Absorption Features with Chemical Data*

Figure 4 shows that there is a good linear relation between the optical densities of the acetate $C-CH_3$ absorption at 1365 $cm^{-1}$ and the chemically estimated acetyl contents in the range of acetyl contents available (0·3 to 5·9 m-equiv/g). The general scatter is within the limits expected from the experimental reproducibility of sample preparation and of chemical estimation of acetyl content. Other absorption features at 1190 and 1755 $cm^{-1}$, characteristic of the acetate group, show the same behaviour (this follows from Fig. 3). The 1365 $cm^{-1}$ ($C-CH_3$) and 1755 $cm^{-1}$ (C=O) bands are the most reliable for quantitative purposes since there is little interference in these regions from absorption due to the coal or char. The 1190 $cm^{-1}$ (C—O) band occurs in a region where coals themselves absorb strongly. A modification of such absorption would be expected to occur on acetylation, owing to the replacement of phenolic hydroxyl groups by phenolic acetate groups ; the phenoxy as well as the acetate

* Unless otherwise stated, all optical densities refer to a 1 per cent. 100 mg disk basis (standard optical density).

TABLE 1

STANDARD OPTICAL DENSITIES OF COALS, CHARS, AND THEIR ACETYL DERIVATIVES

| Source | No. | Sample | Acetyl Content (m-equiv/g) | Optical Densities (1%/100 mg disk basis) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 1365 $cm^{-1}$ ($C—CH_3$) | 1755 $cm^{-1}$ (C=O) | 1190 $cm^{-1}$ (C—O) | 2920 $cm^{-1}$ (C—H) | 3380 $cm^{-1}$ (OH) | 1615 $cm^{-1}$ |
| Yallourn brown coal series | 1 | Coal .. .. .. | | 0·013 | 0·013 | 0·052 | 0·098 | 0·420 | 0·441 |
| | 2 | Coal (acetd.) .. .. | 5·46, 5·39 | 0·316 | 0·615 | 0·826 | 0·123 | 0·145 | 0·330 |
| | 3 | 250 °C char .. .. | | 0·010 | 0·012 | 0·053 | 0·103 | 0·403 | 0·451 |
| | 4 | 250 °C char (acetd.) | 5·48, 5·40 | 0·251 | 0·495 | 0·684 | 0·082 | 0·107 | 0·356 |
| | 5 | 300 °C char .. .. | | 0·010 | 0·016 | 0·080 | 0·098 | 0·318 | 0·463 |
| | 6 | 300 °C char (acetd.) | 5·12, 5·12 | 0·265 | 0·517 | 0·696 | 0·082 | 0·080 | 0·312 |
| | 7 | 350 °C char .. .. | | 0·010 | 0·007 | 0·083 | 0·083 | 0·274 | 0·460 |
| | 8 | 350 °C char (acetd.) | 4·83, 4·63 | 0·262 | 0·495 | 0·763 | 0·070 | 0·085 | 0·344 |
| | 9 | 400 °C char .. .. | | 0·00 | 0·00 | 0·073 | 0·025 | 0·271 | 0·440 |
| | 10 | 400 °C char (acetd.) | 3·31, 3·57 | 0·178 | 0·337 | 0·567 | 0·033 | 0·142 | 0·360 |
| | 11 | 450 °C char .. .. | | — | — | — | 0·014 | 0·239 | 0·387 |
| | 12 | 450 °C char (acetd.) | 1·83, 1·63 | 0·093 | 0·159 | 0·293 | 0·022 | 0·172 | 0·376 |
| | 13 | 500 °C char .. .. | | — | 0·013 | 0·048 | 0·013 | 0·193 | 0·309 |
| | 14 | 500 °C char (acetd.) | 0·57, 0·60 | 0·035 | 0·035 | 0·116 | 0·023 | 0·13 | 0·338 |
| Morwell brown coal series | 15 | Coal .. .. .. | | 0·014 | 0·014 | 0·065 | 0·118 | 0·301 | 0·361 |
| | 16 | Coal (acetd.) .. .. | 5·42, 5·41 | 0·296 | 0·583 | 0·805 | 0·105 | 0·105 | 0·298 |
| | 17 | 300 °C char (acetd.) | 5·25, 5·03 | 0·275 | 0·571 | 0·772 | 0·10 | 0·13 | 0·274 |
| | 18 | 350 °C char (acetd.) | 4·77, 4·54 | 0·239 | 0·494 | 0·675 | 0·093 | 0·14 | 0·280 |
| | 19 | 400 °C char (acetd.) | 3·69, 3·45 | 0·206 | 0·410 | 0·646 | 0·058 | 0·145 | 0·294 |
| | 20 | 500 °C char (acetd.) | 0·36, 0·55 | 0·046 | 0·086 | 0·165 | — | — | 0·300 |
| Alkali-treated* Yallourn brown coal series | 21 | Coal .. .. .. | | 0·011 | 0·005 | 0·115 | 0·122 | 0·38 | 0·380 |
| | 22 | Coal (acetd.) .. | 5·71, 5·90 | 0·309 | 0·595 | 0·900 | 0·134 | 0·014 | 0·248 |
| | 23 | 200 °C char .. .. | | 0·011 | — | 0·127 | 0·121 | 0·25 | 0·385 |
| | 24 | 200 °C char (acetd.) | 5·14, 4·92 | 0·305 | 0·597 | 0·909 | 0·126 | 0·014 | 0·272 |
| | 25 | 250 °C char .. .. | | 0·015 | 0·020 | 0·131 | 0·118 | 0·30 | 0·388 |

* For details of alkali treatments see Brooks, Durie, and Sternhell (1958*b*).

TABLE 1 (*Continued*)

| Source | No. | Sample | Acetyl Content (m-equiv/g) | Optical Densities (1%/100 mg disk basis) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 1365 $cm^{-1}$ ($C—CH_3$) | 1755 $cm^{-1}$ (C=O) | 1190 $cm^{-1}$ (C—O) | 2920 $cm^{-1}$ (C—H) | 3380 $cm^{-1}$ (OH) | 1615 $cm^{-1}$ |
| Alkali-treated* Yallourn brown coal series (*Continued*) | 26 | 250 °C char (acetd.) | 5·01, 5·01 | 0·288 | 0·585 | 0·861 | 0·109 | 0·09 | 0·218 |
| | 27 | 300 °C char .. .. | | 0·016 | 0·023 | 0·113 | 0·106 | 0·22 | 0·358 |
| | 28 | 300 °C char (acetd.) | 4·90, 4·87 | 0·252 | 0·520 | 0·782 | 0·108 | 0·096 | 0·229 |
| | 29 | 350 °C char .. .. | | 0·011 | 0·011 | 0·129 | 0·098 | 0·18 | 0·355 |
| | 30† | 350 °C char (acetd.) | 4·16, 4·11 | 0·170 | 0·364 | — | 0·076 | 0·127 | 0·223 |
| | 31 | 400 °C char .. .. | | — | — | — | 0·039 | 0·18 | 0·287 |
| | 32† | 400 °C char (acetd.) | 3·75, 3·80 | 0·140 | 0·260 | 0·406 | 0·036 | 0·08 | 0·200 |
| | 33 | 460 °C char .. .. | | — | — | — | 0·028 | 0·12 | 0·271 |
| | 34 | 460 °C char (acetd.) | 1·00, 0·97 | 0·074 | 0·105 | 0·240 | 0·06 (?) | 0·14 (?) | 0·263 |
| | 35 | 500 °C char .. .. | | — | — | 0·052 | — | — | 0·268 |
| | 36 | 500 °C char (acetd.) | 0·32 | 0·030 | 0·024 | 0·095 | — | — | 0·298 |
| | 37 | 300 °C char+alkali | | 0·017 | 0·004 | — | 0·109 | 0·32 | 0·346 |
| | 38 | 300 °C char+alkali* (acetd.) | 5·80, 5·60 | 0·248 | 0·488 | 0·676 | 0·131 | 0·19 | 0·251 |
| | | (% C) (% H) | | | | | | | |
| Bituminous coals‡ | 39 | 84·0 6·2 | | 0·016 | — | 0·007 | 0·115 | 0·168 | 0·279 |
| | 40 | Above acetd. | 2·34, 2·34 | 0·137 | 0·210 | 0·310 | 0·135 | 0·192 | 0·233 |
| | 41 | 84·7 5·7 | | 0·025 | — | — | 0·138 | 0·209 | 0·339 |
| | 42 | Above acetd. | 2·17, 2·23 | 0·100 | 0·152 | 0·236 | 0·085 | 0·193 | 0·232 |
| | 43 | 85·7 5·1 | | — | — | — | 0·090 | 0·06 | 0·264 |
| | 44 | Above acetd. | 1·93, 1·77 | 0·118 | 0·172 | 0·280 | 0·134 | 0·09 | 0·319 |
| | 45 | 86·9 2·7 | | 0·018 | — | — | 0·123 | 0·155 | 0·267 |
| | 46 | Above acetd. | 1·43, 1·20 | 0·084 | 0·129 | 0·191 | 0·136 | 0·155 | 0·265 |
| | 47 | 89·9 4·9 | | 0·011 | — | — | 0·102 | 0·119 | 0·269 |
| | 48 | Above acetd. | 0·52, 0·53 | 0·051 | 0·058 | 0·120 | 0·100 | 0·114 | 0·250 |

* For details of alkali treatments see Brooks, Durie, and Sternhell (1958*b*).

† These samples showed some evidence of hydrolysis.

‡ All data for bituminous coals expressed on a dry ash-free basis.

C—O groups of the phenolic-type acetates formed probably contribute jointly in this region. Interference would be expected from further phenoxy absorption if the residual unacetylated hydroxyl groups proved to be phenolic. The correlation shown in Figure 3 between the absorption at 1190 $cm^{-1}$ and that at 1365 $cm^{-1}$ is therefore surprising.

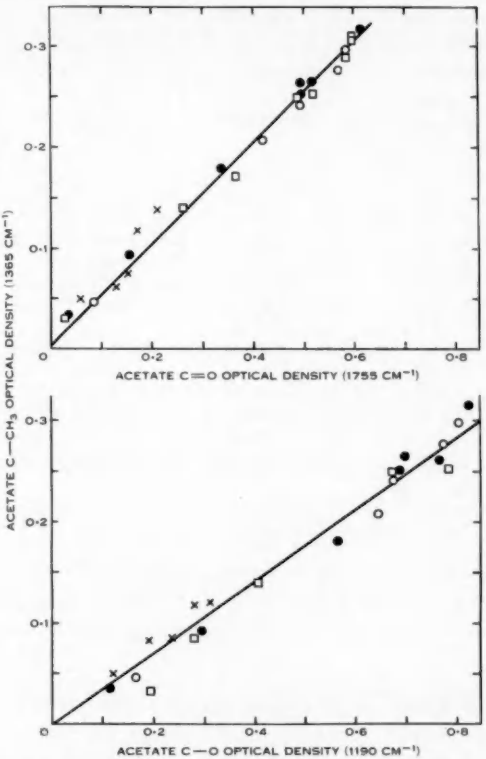


Fig. 3.—Internal comparison of acetate group absorption features (all optical densities on a 1 per cent. 100 mg KCl disk basis).
● Yallourn series ; ○ Morwell series ; □ alkali-treated Yallourn series ; × bituminous coals.

### (c) *Changes in the 2920 $cm^{-1}$ (Aliphatic CH Stretching Vibration) Region on Acetylation*

Brooks, Durie, and Sternhell (1958*a*) noted that no definite changes appear to occur at 2920 $cm^{-1}$ on acetylation of brown coals, in contrast to the appearance of pronounced C—$CH_3$ absorption at 1365 $cm^{-1}$. In the present quantitative studies the changes observed in the optical density at 2920 $cm^{-1}$ on acetylation

were always small and could be either positive or negative depending upon the sample (Table 2).

In attempting to account for this, two factors must be considered—the expected increase in the aliphatic CH absorption due to the introduction of the acetate methyl groups, and the weight factor or dilution effect of the acetate group as a whole. The resultant effect on the strength of the aliphatic CH absorption of these two factors is controlled by the relative specific absorption coefficients (i.e. absorption per CH group) of the aliphatic CH groups in the coal or char samples and of the acetate CH groups introduced.

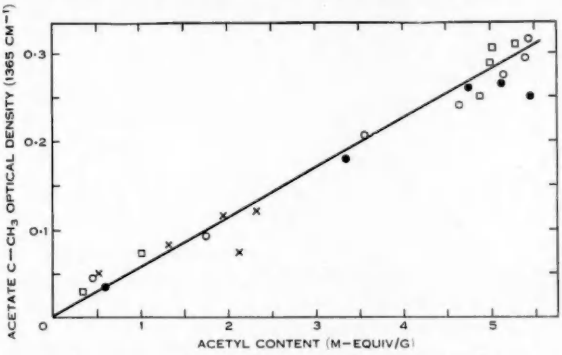


Fig. 4.—Dependence of acetate C—$CH_3$ optical density (at 1365 $cm^{-1}$) on acetyl content.
● Yallourn series; ○ Morwell series; □ alkali-treated Yallourn series; × bituminous coals.

If Beer's law applies to the absorption at 2920 $cm^{-1}$ for the aliphatic CH present in coal, char, and acetate groups using KCl disks, then the optical density $D_a$ of an acetylated sample at 2920 $cm^{-1}$ can be expressed by the formula

$$D_a = D_c.W + A.K, \quad \ldots\ldots\ldots\ldots\ldots\ (1)$$

where $D_c$ = optical density of the original sample (1 per cent./100 mg disk basis);
$A$ = acetyl content of acetylated sample (m-equiv/g);
$W$ = weight factor due to acetylation ($=1-0{\cdot}042A$); and
$K$ = specific absorption coefficient of acetate CH absorption at 2920 $cm^{-1}$.

Since the acetate group absorption appears to be little affected by environment (Fig. 3) a value of $K=0{\cdot}0011$/m-equiv of acetyl groups obtained with KCl disks of dihydroxy diphenyl diacetate was considered satisfactory. The optical densities observed and calculated from equation (1) are compared in Table 2, which shows that the weight factor predominates. It can be seen that, almost without exception, the differences are within the limits of experimental scatter for the 2920 $cm^{-1}$ region (i.e. about $\pm 15$ per cent., owing to the low background energy and weak CH absorption). Thus the observed negligible changes in the 2920 $cm^{-1}$ CH band due to acetylation are in quantitative agreement with the above calculations.

The appearance of the strong acetate C—$CH_3$ absorption at 1370 $cm^{-1}$ in the spectra of acetylated samples is due to the very high specific absorption intensity of the $CH_3$ symmetrical deformation vibration relative to the absorption intensity of the $CH_3$ stretching vibrations for an acetate group. The reverse is true for paraffinic $CH_3$ groups (Francis 1951).

TABLE 2

COMPARISON OF CALCULATED AND OBSERVED ALIPHATIC CH STANDARD OPTICAL DENSITIES AT 2920 $cm^{-1}$ IN ACETYLATED COALS AND CHARS

| Source | No. | Sample | $D_c$ | Acetyl Content $A$ (m-equiv/g) | Weight Factor $W$ (=1—0·42$A$) | $D_a$ | |
|---|---|---|---|---|---|---|---|
| | | | | | | Calc. | Obs. |
| Brown coal series | 1 | Yallourn brown coal | 0·098 | 5·43 | 0·772 | 0·082 | 0·123 |
| | 3 | Yallourn 250 °C char | 0·103 | 5·44 | 0·772 | 0·085 | 0·082 |
| | 5 | Yallourn 300 °C char | 0·098 | 5·12 | 0·785 | 0·081 | 0·082 |
| | 7 | Yallourn 350 °C char | 0·083 | 4·73 | 0·801 | 0·072 | 0·070 |
| | 9 | Yallourn 400 °C char | 0·025 | 3·44 | 0·855 | 0·025 | 0·033 |
| | 15 | Morwell brown coal | 0·118 | 5·42 | 0·772 | 0·098 | 0·105 |
| Alkali-treated Yallourn brown coal series | 21 | Coal | 0·122 | 5·80 | 0·756 | 0·099 | 0·134 |
| | 23 | 200 °C char | 0·121 | 5·03 | 0·789 | 0·091 | 0·126 |
| | 25 | 250 °C char | 0·118 | 5·01 | 0·790 | 0·099 | 0·109 |
| | 27 | 300 °C char | 0·106 | 4·89 | 0·795 | 0·090 | 0·108 |
| | 29 | 350 °C char | 0·098 | 4·14 | 0·826 | 0·086 | 0·076 |
| | 31 | 400 °C char+alkali | 0·039 | 3·78 | 0·841 | 0·037 | 0·036 |
| Bituminous coals | | (% C) (% H) | | | | | |
| | 39 | 84·0 6·2 | 0·115 | 2·34 | 0·899 | 0·129 | 0·135 |
| | 41 | 84·7 5·7 | 0·138 | 2·20 | 0·906 | 0·149 | 0·085 |
| | 43 | 85·7 5·1 | 0·090 | 1·85 | 0·921 | 0·103 | 0·134 |
| | 45 | 86·9 2·7 | 0·123 | 1·31 | 0·944 | 0·130 | 0·136 |
| | 47 | 89·9 4·9 | 0·102 | 0·52 | 0·977 | 0·105 | 0·114 |

### (*d*) *Changes in the 3380 $cm^{-1}$ (OH Stretching Vibration) Region on Acetylation and Pyrolysis*

The spectra of all the acetylated coals and chars covered by the investigation showed pronounced residual OH absorption at 3380 $cm^{-1}$, indicating the presence of bonded hydroxyl groups resistant to acetylation. No significant change was observed in the shape or position of the hydroxyl band on acetylation, so that hydrogen bonding does not appear to be a factor controlling the reactivity of the OH group in coal.

The potassium halide disk technique is not very suited to quantitative infra-red studies in the OH stretching vibration region, since the spectra are susceptible to interference from adsorbed moisture in the disks, and hydrogen bonding of the hydroxyl groups causes additional complications. The hydroxyl absorption intensity $K$ varies with the mode of hydrogen bonding and with type—that is, phenols, alcohols, or carboxylic acids. However, despite these difficulties, when possible moisture interference is neglected and an average $K$

value is assumed for all the hydroxyl groups in coals and chars, the calculations based on the changes in the OH absorption (at 3380 $cm^{-1}$) are remarkably in accord for both the pyrolysed and the acetylated coals.

The chemically non-reactive hydroxyl content of a brown coal can be calculated from a correlation of the change in the OH optical density on acetylation with the change in acetyl content.

Thus, from acetylation changes (assuming Beer's law is valid),

$$D_c = K(H+N+C),$$

and

$$D_a = KW(N+C),$$

so that

$$N = \frac{D_a(H+C) - W.D_c.C}{W.D_c - D_a}, \quad \ldots\ldots\ldots\ldots \quad (2)$$

where $N$ = non-acetylated hydroxyl content of coal (m-equiv/g);
$H$ = acetylated hydroxyl content of coal (m-equiv/g);
$C$ = carboxyl content (m-equiv/g);
$W$ = weight factor due to acetylation ($=1-0\cdot042A$);
$A$ = acetyl content of acetylated coal (m-equiv/g);
$D_a$ = standard optical density at 3380 $cm^{-1}$ for acetylated coal;
$D_c$ = standard optical density at 3380 $cm^{-1}$ for coal; and
$K$ = " averaged " specific absorption coefficient for all hydroxyl groups in coal.

Alternatively, a correlation may be made of the change in OH optical density with the change in content of reactive hydroxyl groups (as determined by acetylation) on pyrolysis at temperatures of the order of 450 °C or less, since for the brown coals studied (Brooks, Durie, and Sternhell 1958*b*) the non-reactive oxygen is known to be stable to 450 °C. Thus, from pyrolysis,

$$D_c = K(H+N+C),$$

and

$$D_p = K(H_p + Nf^{-1} + C_p),$$

giving

$$N = \frac{D_p(H+C) - D_c(H_p+C_p)}{D_c f^{-1} - D_p}, \quad \ldots\ldots\ldots\ldots \quad (3)$$

where, in addition to the notation already given,

$H_p$ = acetylated hydroxyl content of the char;
$C_p$ = carboxyl content of the char;
$D_p$ = standard optical density at 3380 $cm^{-1}$ for the char; and
$f$ = weight factor due to pyrolysis, (100 − % volatiles)/100.

The calculation of the non-reactive hydroxyl content in coal or char by the acetylation method is expected to give the most reliable results for coal and low-temperature chars which have a large reactive hydroxyl content; whereas

TABLE 3

CALCULATED CONTENTS OF NON-ACETYLATABLE HYDROXYL GROUPS IN BROWN COAL AND BROWN COAL CHARS

| | No. | Sample | Optical Density | | Carboxyl Content $C$ (m-equiv/g) | Acetylatable OH Content $H$ (m-equiv/g) | Weight Factor Due to | | Non-Acetylatable OH | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Coal $D_c$ | Acetylated Coal $D_a$ | | | Acetylation $W$ | Pyrolysis $f$ | Calc. (m-equiv/g) | Average |
| (*a*) From changes in OH (3380 $cm^{-1}$) optical density on acetylation | 15 | Morwell brown coal . . . . | 0·301 | 0·105 | 1·33 | 7·00 | 0·774 | 1·00 | 4·44 | |
| | 1 | Yallourn brown coal . . . . | 0·420 | 0·145 | 1·22 | 7·03 | 0·771 | 1·00 | 4·48* | |
| | 3 | Yallourn 250 °C char . . | 0·403 | 0·107 | 0·71 | 7·05 | 0·779 | 0·943 | 2·75 | |
| | 5 | Yallourn 300 °C char . . | 0·318 | 0·080 | 0·22 | 6·51 | 0·787 | 0·915 | 2·61 | 2·8 |
| | 7 | Yallourn 350 °C char . . | 0·274 | 0·085 | — | 5·79 | 0·817 | 0·828 | 2·94 | |
| | | | Char $D_p$ | | $C_p$ | $H_p$ | | | | |
| (*b*) From changes in OH (3380 $cm^{-1}$) optical density on pyrolysis | 9 | Yallourn 400 °C char . . | 0·271 | | — | 4·06 | | 0·726 | 2·72 | |
| | 11 | Yallourn 450 °C char . . | 0·239 | | — | 1·86 | | 0·668 | 3·08 | 2·9 |
| | 13 | Yallourn 500 °C char . . | 0·198 | | — | 0·60 | | 0·604 | 2·80 | |

* These high values probably reflect the presence of adsorbed moisture in the coals which is removed on pyrolysis (cf. Brooks, Durie, and Sternhell 1958*b*). They have therefore been ignored in calculating the averaged value shown.

computations based on equation (**3**) are most reliable for higher-temperature chars. The non-acetylated hydroxyl content calculated by the acetylation method (eqn. (**2**)) for Morwell brown coal, Yallourn brown coal, and the 250, 300, and 350 °C Yallourn chars, are given in (*a*) of Table 3 ; the results of the calculations by the pyrolysis method (eqn. (**3**)) for the 400, 450, and 500 °C chars from Yallourn brown coal are shown in (*b*) of Table 3. The averaged content of

TABLE 4

COMPARISON OF CALCULATED AND OBSERVED STANDARD OPTICAL DENSITIES AT 1615 $cm^{-1}$ IN ACETYLATED COALS AND CHARS

| Source | Sample | Acetyl (m-equiv/g) | Optical Density of Coal $D_c$ | Optical Density of Acetylated Coal | | |
|---|---|---|---|---|---|---|
| | | | | Obs. | Calc. | Difference |
| Brown coal series | Morwell brown coal | 5·41 | 0·361 | 0·298 | 0·279 | —0·019 |
| | Yallourn brown coal | 5·42 | 0·441 | 0·330 | 0·341 | +0·011 |
| | Yallourn 250 °C char | 5·44 | 0·451 | 0·356 | 0·348 | —0·008 |
| | Yallourn 300 °C char | 5·12 | 0·463 | 0·312 | 0·363 | —0·051 |
| | Yallourn 350 °C char | 4·73 | 0·460 | 0·344 | 0·368 | —0·024 |
| | Yallourn 400 °C char | 3·44 | 0·440 | 0·360 | 0·376 | —0·016 |
| | Yallourn 450 °C char | 1·73 | 0·387 | 0·376 | 0·360 | +0·016 |
| | Yallourn 500 °C char | 0·58 | 0·309 | 0·289 | 0·301 | —0·012 |
| Alkali-treated Yallourn brown coal series | Coal .. .. | 5·80 | 0·380 | 0·248 | 0·287 | +0·039 |
| | 200 °C char .. | 5·03 | 0·385 | 0·272 | 0·303 | +0·031 |
| | 250 °C char .. | 5·01 | 0·388 | 0·218 | 0·306 | +0·088 |
| | 300 °C char .. | 4·88 | 0·358 | 0·229 | 0·284 | +0·055 |
| | 350 °C char .. | 4·13 | 0·355 | 0·223 | 0·293 | +0·070 |
| | 400 °C char .. | 3·78 | 0·287 | 0·200 | 0·241 | +0·041 |
| | 460 °C char .. | 0·99 | 0·271 | 0·263 | 0·260 | —0·003 |
| | 500 °C char .. | 0·32 | 0·268 | 0·298 | 0·264 | —0·034 |
| | 300 °C char+alkali* | 5·7 | 0·346 | 0·251 | 0·263 | +0·012 |
| | (% C) (% H) | | | | | |
| Bituminous coals | 84·0 6·2 | 2·34 | 0·279 | 0·233 | 0·251 | +0·018 |
| | 84·7 5·7 | 2·20 | 0·339(?) | 0·232 | 0·307 | +0·075 |
| | 85·7 5·1 | 1·85 | 0·264 | 0·319 | 0·243 | —0·076 |
| | 86·9 2·7 | 1·31 | 0·267 | 0·265 | 0·252 | —0·013 |
| | 89·9 4·9 | 0·52 | 0·269 | 0·250 | 0·263 | +0·013 |

* For details of the alkali treatments see Brooks, Durie, and Sternhell (1958*b*).

non-acetylated hydroxyl content in both cases is about 3 m-equiv/g. The agreement between the two methods is surprising in view of the approximate nature of the calculations and the experimental difficulties involved.

Thus it would appear that in the brown coals under consideration, the hydroxyl groups which are unreactive to acetylation are stable up to at least 450 °C. The calculated amount of such groups (about 3 m-equiv/g) present is very near to the content of non-reactive oxygen groups in Victorian brown coals,

estimated previously by chemical studies (Brooks and Sternhell 1957). This suggests that the estimated 5 to 7 per cent. of non-reactive oxygen in these brown coals is predominantly in the form of non-reactive hydroxyl groups.

### (e) *Effect of Acetylation on the 1600 cm$^{-1}$ Band in Coal*

Brooks, Durie, and Sternhell (1958*a*) noted an apparent decrease in the strength of the " 1600 cm$^{-1}$ " band in the spectra of brown coals on acetylation. This observation was confirmed in the present study. However, the change has now been found to be predominantly due to the weight factor—that is, to the decrease in the actual mass of such structures contributing to this absorption in a standard disk containing the acetylated coal. The calculated standard optical densities of the acetylated samples obtained by multiplying the standard optical densities $D_c$ of the original samples by the weight factor $W$ ($=1-0{\cdot}042\times$acetyl content in m-equiv/g) are in good agreement with the observed standard optical densities (Table 4) when due allowance is made for experimental scatter.

Thus the acetylation of hydroxyl groups in the coals studied does not appear to cause structural (chemical) changes which affect the 1600 cm$^{-1}$ absorption.

It is possible that the reported decrease in the 1600 cm$^{-1}$ band on reductive acetylation, for a British bituminous coal (Brown and Wyss 1955), was due to the same effect.

## IV. Acknowledgments

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# 2- AND 3-PHENYLTHIONAPHTHENS

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*Summary*

The product obtained from 3-(1′-*cyclo*hexenyl)thionaphthen (I) by dehydrogenation with sulphur at 240–250 °C is shown to be 2-phenylthionaphthen rather than 3-phenylthionaphthen (II) as assumed by Schuetz and Ciporin (1958*a*, 1958*b*). The dissimilarity of the ultraviolet absorption spectra of 2- and 3-phenylthionaphthens is discussed.

## I. INTRODUCTION

3-Arylthionaphthens usually have much lower melting points than the corresponding 2-aryl compounds; for example 5-methyl-3-phenylthionaphthen is an oil whereas the 5-methyl-2-phenyl compound is a solid, m.p. 158–158·5 °C (Banfield *et al.* 1956). Therefore, since 2-phenylthionaphthen is known to be a solid, m.p. 175–176 °C (Horton 1949; Banfield *et al.* 1956; Dann and Kokurudz 1958), it would be expected that 3-phenylthionaphthen (II) would have a melting point lower than that (172–173 °C) reported by Schuetz and Ciporin (1958*a*, 1958*b*) for their "3-phenylthionaphthen", which was obtained from 3-(1′-*cyclo*hexenyl)thionaphthen (I) by dehydrogenation with sulphur at 240–250 °C. Indeed, the closeness of the m.p. of Schuetz and Ciporin's product with that of 2-phenylthionaphthen strongly suggested that rearrangement had accompanied the dehydrogenation reaction, and this suggestion received support when it was found that the ultraviolet absorption spectrum reported by Schuetz and Ciporin for their 3-phenylthionaphthen is essentially the same as that of the 2-phenyl compound. To finalize the issue, however, an alternative synthesis of 3-phenylthionaphthen (II) was undertaken.†

## II. THE SYNTHESIS OF 3-PHENYLTHIONAPHTHEN

2-Chloro 5-nitrobenzophenone (V) was prepared from benzene and 2-chloro-5-nitrobenzoyl chloride by the Friedel-Craft reaction. It was then converted into *S*-2-benzoyl-4-nitrophenylmercaptoacetic acid (VI) by reaction with sodium mercaptoacetate (thioglycollate). Ring closure with aqueous potassium hydroxide gave 5-nitro-3-phenylthionaphthen-2-carboxylic acid (III) which was reduced to the corresponding amino compound by use of ferrous sulphate and ammonia. Deamination then gave 3-phenylthionaphthen-2-carboxylic acid which was decarboxylated using copper chromite and quinoline to give 3-phenyl-

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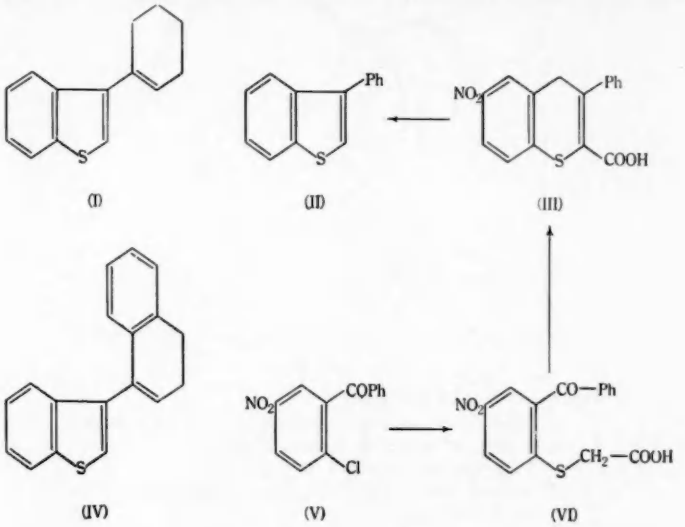
† Dann and Kokurudz (1958) have reported that cyclization of phenylphenacyl sulphide with polyphosphoric acid at 100 °C gives 3-phenylthionaphthen, but, since the sulphide is converted into 2-phenylthionaphthen by the same reagent at 180 °C (Banfield *et al.* 1956), it seems probable that the product obtained by Dann and Kokurudz was actually a mixture of the two isomeric phenylthionaphthens.

thionaphthen (II) as an oil (as expected), the ultraviolet absorption spectrum of which was different from that of Schuetz and Ciporin's 3-phenylthionaphthen.

It is therefore established that rearrangement has accompanied the dehydrogenation of I, from which it follows that the product obtained by sulphur-dehydrogenation of 3-(3′,4′-dihydro-1′-naphthyl)thionaphthen (IV) is not necessarily 3-(1′-naphthyl)thionaphthen as reported by Schuetz and Ciporin (1958*a*, 1958*b*); indeed, the product may well be the corresponding 2-(1′-naphthyl)thionaphthen. It also follows that the conclusions drawn by Schuetz and Ciporin from a comparison of the spectrum of their "3-phenylthionaphthen" with those of several other 3-arylthionaphthens are invalid.




### III. The Ultraviolet Spectra of 2- and 3-Phenylthionaphthens

As may be seen from Figure 1, the ultraviolet absorption spectra of 2- and 3-phenylthionaphthens are markedly dissimilar, a phenomenon which may be explained by considering the planar projection diagrams of the two compounds and then following a line of argument analogous to that used by Friedel, Orchin, and Reggel (1948) to explain the dissimilarity of the spectra of 1- and 2-phenylnaphthalenes. Thus, in the case of 2-phenylthionaphthen, the projection diagram (Fig. 2 (*a*)) shows that the phenyl group is unhindered, so that coplanarity and effective internuclear conjugation are possible. As a result, the absorption spectrum is markedly different from that of thionaphthen.*

* Strictly speaking, the spectra of the phenylthionaphthens should be compared with the curve obtained by superimposing the spectra of thionaphthen and benzene rather than with the spectrum of thionaphthen itself. However, since benzene shows only relatively weak absorption in the wavelength range being considered, this composite spectrum is not significantly different from that of thionaphthen itself.

In the case of the 3-phenyl compound, however, the projection diagram (Fig. 2 (*b*)) clearly shows that steric interaction between the *o*-hydrogen atoms of the phenyl group and the 4-hydrogen atom of the thionaphthen nucleus would tend to hinder coplanarity, thereby tending to reduce the extent of internuclear conjugation, with the result that the absorption spectrum resembles that of thionaphthen.

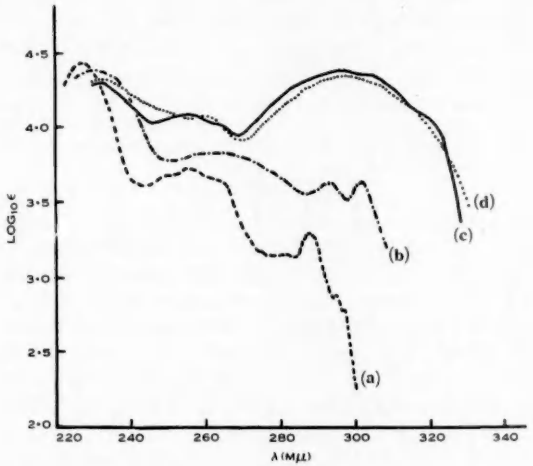



Fig. 1.—Ultraviolet absorption spectra.

(*a*) Thionaphthen in 95% ethanol (Badger and Christie 1956).
(*b*) 3-Phenylthionaphthen in 95% ethanol.
(*c*) 2-Phenylthionaphthen in 95% ethanol.
(*d*) Schuetz and Ciporin's "3-phenylthionaphthen" in *cyclo*hexane.

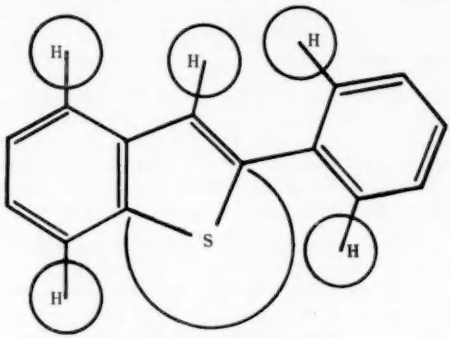
However, although this explanation seems quite reasonable, it is probably not the complete explanation since it is likely that introduction of even a simple substituent into the hetero-ring of thionaphthen would have a different effect on the absorption spectrum according as the substituent entered the 2- or the 3-position, just as the effect produced by introducing a simple substituent into the benzenoid ring varies with the position of substitution (Padhye and Desai 1953). In the case of thiophen, introduction of a substituent into the 2-position usually has a greater effect on the absorption spectrum than does introduction of the same substituent into the 3-position,* and a similar relationship may exist between 2- and 3-substituted thionaphthens. Unfortunately, no data are available to enable a test to be made.

* For example, Elpern and Nachod (1950) have shown that 2-phenylthiophen absorbs at longer wavelengths than the 3-phenyl compound, even though projection diagrams, such as Figures 2 (*a*) and 2 (*b*), readily show that molecular coplanarity is possible in both isomers.

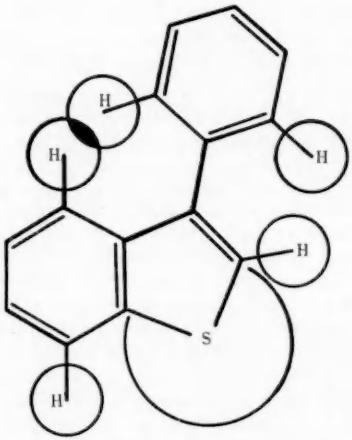
## IV. EXPERIMENTAL

Melting points are uncorrected. Analyses are by the C.S.I.R.O. Microanalytical Laboratory. A Hilger Uvispeck spectrophotometer was used to determine ultraviolet absorption spectra.

(*a*) *2-Chloro-5-nitrobenzophenone* (*V*).—2-Chloro-5-nitrobenzoic acid (5·0 g), prepared by nitration of *o*-chlorobenzoic acid as described by Lehmstedt (1931), was treated under reflux




(*a*) 2-Phenylthionaphthen




(*b*) 3-Phenylthionaphthen

Fig. 2.—Planar projection diagrams.

In constructing these diagrams it has been assumed : (i) That the bond angles and lengths in the hetero-rings of the thionaphthenyl nuclei are essentially the same as in thiophen (Schomaker and Pauling 1939 ; see also Bak *et al.* 1956 ; Crumper 1958) ; (ii) that the bond angles and lengths in the benzenoid rings of the thionaphthenyl nuclei, and also in the phenyl groups, are the same as in benzene (Coulson 1952) ; (iii) that the C—H bonds are of length 1·09 Å (Crombie 1955) ; (iv) that the compounds are coaxial ; (v) that the interannular bond length is 1·48 Å, the same as in diphenyl (Dhar 1932) ; (vi) that the van der Waal's radius of the hydrogen atom is 0·75 Å (Crombie 1955) ; (vii) that the van der Waal's radius of the sulphur atom is 1·7 Å (Knott 1955).

with excess thionyl chloride (10 ml) for 2 hr, the excess removed by distillation, and the oily residue taken up in dry benzene (30 ml). Powdered aluminium chloride (5·0 g) was then added portionwise during 30 min, the exothermic reaction being moderated by external cooling. The reaction mixture was then treated under reflux for 1·5 hr, cooled, poured into an ice/HCl mixture, and the organic layer was separated and washed with water, dilute NaOH, and again with water. It was then dried ($MgSO_4$) and evaporated to give a brown solid which was recrystallized from ethanol to give the required benzophenone derivative (5·3 g, 81%), m.p. 85·5–86 °C; cf. lit. m.p. 86 °C (Ullmann and Mallet 1898; Fries 1927), m.p. 89 °C (Meisenheimer, Zimmermann, and von Kummer 1926).

(*b*) *5-Amino-3-phenylthionaphthen-2-carboxylic Acid.*—This compound was prepared from V by the method of Angelini (1957). Thus, V was treated with sodium mercaptoacetate to give 2-benzoyl-4-nitrophenylmercaptoacetic acid (VI) which was cyclized with aq. KOH to give 5-nitro-3-phenylthionaphthen-2-carboxylic acid (III), m.p. 249–251 °C (lit. m.p. 250–251 °C), which was then reduced with ferrous sulphate/ammonia to give the required amino compound, m.p. 220–221 °C (lit. m.p. 219–220 °C).

(*c*) *3-Phenylthionaphthen-2-carboxylic Acid.*—The above amine (2·0 g) was dissolved in warm glacial acetic acid (80 ml) and the solution added during 30 min with stirring and cooling to a solution of $NaNO_2$ (0·68 g) in conc. $H_2SO_4$ (8 ml). This mixture was then added during 15 min to a stirred suspension of cuprous oxide (2·1 g, prepared by the method of Palmer 1954) in absolute ethanol (180 ml). After being stirred for a further 10 min, the mixture was diluted with water (*c.* 1200 ml), extracted with ether (200 ml), and this extract was washed with 2N HCl (50 ml), water, and finally with aq. NaOH. This last extract was acidified with conc. HCl, and the resultant precipitate recrystallized from benzene/light petroleum (b.p. 40–60 °C) to give the required *acid* (1·3 g, 70%), colourless prisms, m.p. 199–200 °C (Found: C, 71·1; H, 4·0%; neut. equiv. 251. Calc. for $C_{15}H_{10}O_2S$: C, 70·9; H, 3·9%; neut. equiv. 254).

(*d*) *3-Phenylthionaphthen (II).*—The above acid (1·2 g) was heated with copper chromite (0·2 g) and quinoline (50 ml) at 220–230 °C until evolution of $CO_2$ had ceased (30 min), the mixture was poured into cold dilute HCl, *immediately* extracted with ether, and the ether extract *immediately* washed with 2N NaOH, and then with water. Evaporation of the ether gave a dark brown oil which was dissolved in *cyclo*hexane and chromatographed on alumina. The fraction with weak blue fluorescence in ultraviolet light was collected and evaporated to give the *product* (0·8 g, 82%) as a colourless oil (Found: C, 79·9; H, 4·8; S, 15·0%. Calc. for $C_{14}H_{10}S$: C, 80·0; H, 4·8; S, 15·2%). The ultraviolet absorption spectrum in 95% ethanol (Fig. 1) showed maxima at 231, 263, 293, and 302 mμ (log ε 4·39, 3·83, 3·63, and 3·64 respectively) and minima at 250, 287, and 298 mμ (log ε 3·78, 3·55, and 3·51 respectively). The product gave a burgundy colour with conc. $H_2SO_4$. It did not give a derivative with either picric acid in ethanol, or with 2,4,7-trinitrofluorenone in benzene/absolute ethanol (1 : 1), although an orange colour was produced with the latter reagent. When the product was treated with benzotrifuroxan (Bailey and Case 1958) in absolute ethanol/acetic acid (4 : 1), it gave a *benzotrifuroxan derivative*, yellow plates from ethanol, m.p. 161–161·5 °C (Found: C, 47·3; H, 1·9; S, 5·2; N, 20·7; O, 24·4%. Calc. for $2C_{14}H_{10}S/3C_6O_6N_6$: C, 46·9; H, 1·7; S, 5·5; N, 21·4; O, 24·5%). The formation of a complex of the 2 : 3 type rather than of the normal 1 : 1 type is of interest since the isosteric 1-phenylnaphthalene also gives a 2 : 3 complex with benzotrifuroxan (Bailey and Case 1958).

(*e*) *2-Phenylthionaphthen.*—This compound was obtained from fluorobenzene and 2-thionaphthenyl lithium as already described (Banfield *et al.* 1956). The ultraviolet absorption spectrum in 95% ethanol (Fig. 1) showed maxima at 232, 254, and 297 mμ (log ε 4·32, 4·09, and 4·38 respectively) and minima at 246 and 268 mμ (log ε 4·04 and 3·95 respectively). The compound gave a cinnamon colour with cold conc. $H_2SO_4$. It did not give a derivative with picric acid in ethanol, but with 2,4,7-trinitrofluorenone in benzene/absolute ethanol (1 : 1) it gave a *2,4,7-trinitrofluorenone derivative*, orange microneedles, m.p. 195–195·5 °C (Found: N, 7·8; S, 6·0%. Calc. for $C_{14}H_{10}S/C_{13}H_5N_3O_7$: N, 8·0; S, 6·1%).

## V. Acknowledgments

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# STUDIES OF THE OPTICALLY ACTIVE COMPOUNDS OF ANACARDIACEAE EXUDATES

## V. FURTHER INVESTIGATION OF THE EXUDATE FROM CAMPNOSPERMA AURICULATA HOOK.F.

By J. A. LAMBERTON*

[*Manuscript received October* 30, 1958]

*Summary*

Distillation of the hydrogenated oil from *Campnosperma auriculata* Hook.f. yields *n*-pentacosane-2,6-dione (IV), which is formed from (+)-3-hydroxy-3-nonadecyl*cyclo*hexanone (II) by a retro-aldol reaction. In alcoholic alkali, IV is recyclized to 3-nonadecyl*cyclo*hex-2-enone (III), and on Wolff-Kishner reduction the disemicarbazone of IV yields *n*-pentacosane. Oxidation of 3-nonadecyl*cyclo*hex-2-enone (III) yields 5-ketotetracosanoic acid, which has been synthesized by an improved method. The synthesis of III and of 3-heptadecyl*cyclo*hex-2-enone is described and a convenient method is given for the preparation of 3-methyl*cyclo*hex-2-enone.

Examination of the mixture of phenols obtained by the action of alkali on *C. auriculata* oil has shown the presence of 3-nonadecyl phenol and a mixture composed predominantly of *cis*-monoethylenic $C_{19}$ alkyl phenols. The phenols are admixed with a small amount of certain long-chain quinols which are also present in the original oils. Nonadecylquinol, one component, has been characterized by conversion into nonadecylbenzoquinone.

The exudate from *Campnosperma macrophylla* Hook.f. (also Malayan) is unlike that from *C. auriculata* and contains little oil.

### I. INTRODUCTION

An earlier study of the oily exudate from the Malayan tree *Campnosperma auriculata* Hook.f., described in Part III of this series (Lamberton 1958*a*), showed the presence of optically active long-chain compounds which were assigned the general structure I and differed among themselves only in the amount and location of unsaturation in the $C_{19}$ aliphatic chain. On catalytic hydrogenation these compounds were converted into (+)-3-hydroxy-3-nonadecyl*cyclo*hexanone (II), and in alcoholic alkali they were converted into a mixture of phenols. A further examination has now been made of the same specimen of *Campnosperma auriculata* oil and also of a second sample of oil collected at a later date. The two specimens are closely similar, but differ in some respects.

### II. *n*-PENTACOSANE-2,6-DIONE AND 3-NONADECYLCYCLOHEX-2-ENONE

It has now been found that distillation of the hydrogenated oil at low pressure gives yields as high as 40–50 per cent. by weight of *n*-pentacosane-2,6-dione (IV). The structure IV follows from the facts that the diketone is rapidly cyclized by warm alcoholic alkali to 3-nonadecyl*cyclo*hex-2-enone (III) and that, after

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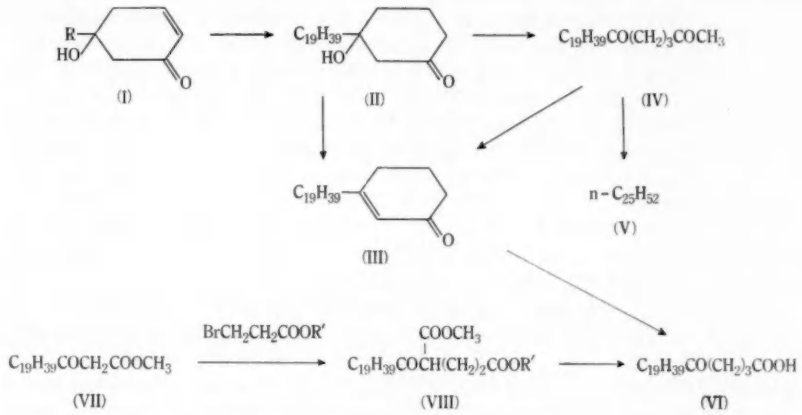
conversion into its disemicarbazone to prevent cyclization, it yields *n*-pentacosane (V) on Wolff-Kishner reduction. Pentacosane-2,6-dione presumably arises from II by a retro-aldol reaction involving ring fission. This pyrolysis reaction is comparable with the reverse Michael fission reactions described in Part IV of this series (Lamberton 1958*b*). Dehydration of II also takes place during the distillation of the hydrogenated oil and 3-nonadecyl*cyclo*hex-2-enone also distils. The latter is easily obtained in quantity by heating the hydrogenated oil in alcoholic alkali for a few minutes before distillation to ensure dehydration of II.




Oxidation of 3-nonadecyl*cyclo*hex-2-enone (III) with potassium permanganate in acetone yields 5-ketotetracosanoic acid (VI), which by Wolff-Kishner reduction is easily converted into tetracosanoic acid. A general method for the preparation of 5-keto acids of this type has been described by Stetter and Dierichs (1952). For example, they prepared 5-ketodocosanoic acid by alkylating the potassium salt of dihydroresorcinol with hexadecyl iodide and then cleaving the resulting monoalkylated diketone. However, the overall yield is low (less than 30 per cent.) as a large proportion of *o*-alkyl ether is produced in the alkylation step and solubility difficulties increase with increasing molecular weight of the alkyl iodide. It is more convenient to prepare methyl 3-ketoeicosanoate from stearoyl chloride and the sodium derivative of acetoacetic ester by the method of Stallberg-Stenhagen and Stenhagen (1944) and then alkylate the keto ester with ethyl β-bromopropionate. Hydrolysis and ketonic cleavage then yields 5-ketodocosanoic acid. This same method applied to methyl 3-ketodocosanoate (VII) gives the alkylation product VIII which is in turn hydrolysed to 5-ketotetracosanoic acid, identical with that obtained by oxidizing III.

Attention has been given to the problem of finding a convenient synthesis of long-chain 3-alkyl*cyclo*hex-2-enones. Birch (1946, 1947) found that reduction of anisole with sodium and alcohol in liquid ammonia yields *cyclo*hex-2-enone, and this reaction normally proceeds satisfactorily with 3-substituted anisoles (e.g. Grewe, Nolte, and Rotzoll 1956). However the reaction is not applicable

to 3-methoxynonadecylbenzene because it is completely insoluble in the reaction mixture. Another procedure due to Woods *et al.* (1949) in which 3-ethoxy-*cyclo*hex-2-enone is added to a Grignard reagent is satisfactory, and 3-heptadecyl- and 3-nonadecyl*cyclo*hex-2-enone were prepared in this way, although the yields are not high. An attempt to convert the 5-keto acids mentioned above into methyl ketones which could then be cyclized to *cyclo*hex-2-enones was unsuccessful because it has not been possible to carry out the normal sequence of making acyl chlorides from 5-keto acids and then condensing with ethoxymagnesiomalonic ester. This may be due to the formation of unsaturated lactones during attempted preparation of the acyl chlorides; it is known that 5-ketohexanoic acid is very easily cyclized to 5-hexenolactone. Although unsuccessful for the long-chain 3-alkyl*cyclo*hex-2-enones, the same principle may be applied to the preparation of 3-methyl*cyclo*hex-2-enone. Glutaryl chloride reacts with ethoxymagnesiomalonic ester to give a high yield of glutaryldimalonic ester, which on heating with acid undergoes simultaneous hydrolysis and cyclization. Fargher and Perkin (1914) observed the cyclization of heptane-2,6-dione to 3-methyl-*cyclo*hex-2-enone.

Although IV is cyclized so readily by warming in alcoholic alkali, when it is heated in alcoholic ammonia solution under the conditions used in the Hantzsch synthesis of dihydropyridines, no cyclization to a dihydropyridine, or to III occurs. The 1,5-diketones which are considered to be intermediates in the Hantzsch synthesis (see Haley and Maitland 1951) always contain carbethoxy groups β to each carbonyl group and this presumably aids cyclization. This agrees with the observation that the condensation of primary amines with 1,4-diketones to give pyrroles by the Knorr-Paal method requires vigorous conditions, but diethyl 3,6-diketo-octanedioate when warmed with aqueous methylamine gives a good yield of 1-methylpyrrole-2,5-diacetic ester (Willstatter and Pfannenstiel 1921). Presumably under sufficiently drastic conditions IV could be converted into a dihydropyridine for Frank and Meikle (1950) have shown that even 3-alkyl*cyclo*hex-2-enones on heating with ammonia and ammonium acetate at 250 °C yield dihydropyridines: in these instances 1,5-diketones produced by a retro-aldol reaction are again postulated as intermediates.

### III. Phenols Derived from C. auriculata Oil

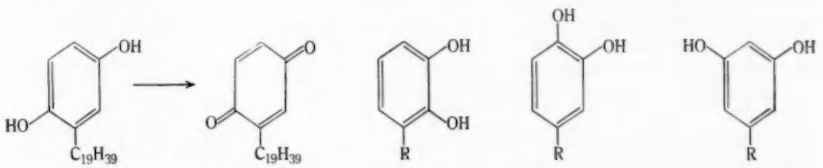
As previously described, the oil from *C. auriculata* is converted into a mixture of phenols when heated in alcoholic alkali. On distillation, these mostly distil over a narrow temperature range, and the distillation temperature rises only while the last 20 per cent. is distilled. When the phenols prepared from the oil used in the earlier investigation are distilled, 3-nonadecyl phenol crystallizes out rapidly from the first fractions collected, but the second batch of oil contains less saturated material. In both instances after methylation of the phenols and subsequent hydroxylation by the performic acid method, chromatography on alumina yields 3-methoxynonadecylbenzene and more strongly absorbed hydroxylated methyl ethers. The latter crystallize readily from light petroleum but are evidently a mixture for the melting point is not sharp, and a mixture of aldehyde 2,4-dinitrophenylhydrazones is obtained by periodate oxidation.

Analysis of the mixture of hydroxylated methyl ethers indicates that there are mostly compounds having only one double bond per molecule in the original mixture of unsaturated methyl ethers, while the infra-red absorption of the methyl ethers shows that the double bonds are *cis* as the absorption at *c.* 960 $cm^{-1}$ known to be characteristic of a *trans*-olefine is absent (Loev and Dawson 1958).

## IV. Long-Chain Quinols

In the distillation of the phenols prepared from the second batch of *C. auriculata* oil there is a significant difference in the ultraviolet absorption spectrum of the later fractions, and the substances responsible for this may be easily separated, being relatively insoluble in light petroleum. When these later fractions are hydrogenated before treatment with light petroleum there is an increased yield of this material, which corresponds to about 2–3 per cent. of the whole oil. This crude material has an ultraviolet absorption ($\lambda_{max.}$ 292 mμ ; ε 3550) characteristic of a quinol and although it crystallizes readily from light petroleum, on attempted crystallization from aqueous ethanol a dark blue colour develops. Oxidation with silver oxide in dry ether gives a mixture of long-chain benzoquinones. When the benzoquinones and quinols are mixed the blue colour develops rapidly and the colour is evidently due to quinhydrone formation ; Cook, Heilbron, and Lewis (1942) have reported the formation of such coloured materials from long-chain quinols. The crude quinols and quinones obtained in this way are obviously mixtures, but isolation of the quinol from fractions of intermediate boiling point, oxidation to the quinone, and purification by chromatography give nonadecylbenzoquinone (X). This establishes that nonadecylquinol (IX) is one component of the mixture of quinols.

The long-chain quinols are not artefacts produced by the action of alkali for they may be isolated by other methods, some of which are more direct. They are present in the higher boiling fractions obtained in the distillation of (*a*) the original oil, (*b*) the original oil after heating with alcoholic hydrochloric acid, and (*c*) the hydrogenated oil. The amount of quinol in the oil used in the earlier studies was very small, and only traces could be separated.




(IX) (X) (XI) (XII) (XIII)

Long-chain quinols have not previously been reported as components of Anacardiaceae exudates, although both catechols and resorcinols have been found. Catechols of the type XI having a $C_{15}$ chain are found in the various oils of the urushiol type, which occur in *Rhus toxicodendron* (Symes and Dawson 1954), *R. vernicifera* (Sunthankar and Dawson 1954), *Semecarpus anacardium*

(Pillay and Siddiqui 1931), and *S. heterophylla* (Backer and Haack 1938); while oils of the same type but having a $C_{17}$ chain occur in *R. succedanea*, *S. vernicifera*, *R. ambigua* (Majima 1922), *Gluta renghas* (Backer and Haack 1941), *Holygarna arnottiana* (Nair, Poti, and Pillay 1952*a*), and *S. travancorica* (Nair, Poti, and Pillay 1952*b*). Moreacol (Backer and Haack 1941) and thitsiol (Majima 1922) from *Melanorrhoea* species have been shown to contain catechols of the type XII. Examples of resorcinols of type XIII are cardol from Cashew nut-shell liquid (Wasserman and Dawson 1948) and bilobol from *Ginkgo biloba* (Kawamura 1928). All these types may be regarded as being formed in the plant by further hydroxylation of the 3-alkyl phenols, the biogenesis of which is discussed in Part I of this series (Dalton and Lamberton 1958).

There are three Malayan *Campnosperma* species, namely *C. minor*, *C. macrophylla*, and *C. auriculata*. The exudate from *C. macrophylla* Hook.f. contains very little oil, which, although optically active, is quite different from that of *C. auriculata*.

## V. Experimental

### (*a*) *General*

As in Part I of this series (Dalton and Lamberton 1958).

The remainder of the specimen of *C. auriculata* oil used in the earlier study and kept in a refrigerator in the interval and a second specimen of the oil having closely similar properties (ultraviolet absorption, optical rotation) were both examined. Unless otherwise specified the same result was obtained with each oil.

### (*b*) n-*Pentacosane-2,6-dione* (*IV*)

*C. auriculata* oil (10 g) was dissolved in methyl acetate (300 ml) and hydrogenated over platinum oxide until uptake of hydrogen ceased after absorption of 1220 c.c. After filtering from the catalyst, the solution was evaporated and the residue distilled at 1–1·5 mm pressure. Almost the whole contents of the flask distilled evenly at 235–240 °C and immediately crystallized. The distillate was sparingly soluble in ether, and was finally dissolved in 600 ml of ether under reflux, and the solution filtered. On standing overnight, n-*pentacosane-2,6-dione* (4·5–5·0 g) separated as colourless needles, m.p. 89–90 °C. This melting point was not improved by recrystallization (Found: C, 79·2; H, 12·5%. Calc. for $C_{25}H_{48}O_2$: C, 78·9; H, 12·6%). This compound showed no significant ultraviolet absorption in ethanolic solution, and only a slight peak at $\lambda$ 238 m$\mu$ when ethanolic potassium hydroxide was added in the cold. After warming for 2 min on a steam-bath the alkaline solution showed $\lambda_{max.}$ 238 m$\mu$; $\varepsilon$ 12,250. The infra-red absorption spectrum showed no hydroxyl and only a strong carbonyl absorption. The carbonyl band (1707 cm$^{-1}$) was asymmetric, having a slight shoulder at 1712 cm$^{-1}$.

Heating IV with ethanolic potassium hydroxide for several minutes gave 3-nonadecyl*cyclo*hex-2-enone, which was identified by the preparation of derivatives (see below).

The *disemicarbazone* from IV was very sparingly soluble in ethanol and was conveniently crystallized from amyl acetate. It gave colourless prisms, m.p. 177–178 °C (Found: C, 65·6; H, 10·9; N, 16·8%. Calc. for $C_{27}H_{54}O_2N_6$: C, 65·6; H, 10·9; N, 17·0%).

The *dioxime* from IV crystallized from slightly aqueous ethanol in colourless prisms, m.p. 75–76 °C (Found: N, 6·5%. Calc. for $C_{25}H_{50}O_2N_2$: N, 6·8%).

An attempt to prepare a 2,4-dinitrophenylhydrazone from IV gave a product which was very difficult to purify. It melted at 103–104 °C (not sharply) after crystallization from ethanol, and the analysis is close to that of the bis-derivative (Found: C, 60·7; H, 7·8; N, 14·6%. Calc. for $C_{37}H_{56}O_8N_8$: C, 60·0; H, 7·6; N, 15·1%).

The disemicarbazone of IV (1·0 g) and excess hydrazine were added to diethylene glycol (50 ml) in which sodium (1·5 g) had been dissolved. After heating at 180 °C for 7 hr the product

was purified by passing a solution in light petroleum through a short column of alumina. Crystallization from ethanol–toluene gave *n*-pentacosane as colourless plates, m.p. 53·5–54 °C (Found : C, 85·1 ; H, 14·8%. Calc. for $C_{25}H_{52}$ : C, 85·2 ; H, 14·8%).

A synthetic specimen of *n*-pentacosane was prepared for comparison by the following route : ketonization of tridecanoic acid by heating with magnesium oxide at 360 °C according to the general procedure of Curtis, Dobson, and Hatt (1947) gave 13-pentacosanone, m.p. 73–74 °C (Meals (1944) reports m.p. 73–74 °C), which on Wolff-Kishner reduction gave *n*-pentacosane, m.p. 53·5–54 °C, identical with that obtained above.

### (c) *3-Nonadecylcyclohex-2-enone (III)*

After separating *n*-pentacosane-2,6-dione from the distillation products of the hydrogenated oil, the ethereal mother liquors were evaporated, and the residue shown to consist mainly of 3-nonadecylcyclohex-2-enone (III), which was isolated by chromatography on alumina. When the hydrogenated oil is heated in alcoholic alkali before distillation, III is almost the sole product.

As previously, III was characterized by its ultraviolet absorption, infra-red absorption, and the preparation of its 2,4-dinitrophenylhydrazone. The *semicarbazone* of III was obtained as colourless prisms, m.p. 141–142 °C, from ethanol (Found : C, 74·2 ; H, 11·9 ; N, 9·8%. Calc. for $C_{26}H_{49}ON_3$ : C, 74·5 ; H, 11·7 ; N, 10·0%). The *oxime* from III crystallized in colourless prisms, m.p. 74–75 °C, from ethanol (Found : C, 79·5 ; H, 12·5 ; N, 3·8%. Calc. for $C_{25}H_{47}ON$ : C, 79·6 ; H, 12·5 ; N, 3·7%).

### (d) *5-Keto Acids*

(i) *Oxidation of III.*—3-Nonadecylcyclohex-2-enone (3·0 g) was dissolved in dry acetone and finely powdered potassium permanganate added to the solution at its boiling point until a violet colour persisted. After crystallization from ethanol the crude acidic product (2·1 g) melted at 90–92 °C. Further crystallization from ethanol gave 5-ketotetracosanoic acid as colourless prisms, m.p. 102–103 °C (Found : C, 75·6 ; H, 12·1%. Calc. for $C_{24}H_{46}O_3$ : C, 75·3 ; H, 12·0%). It yielded a *2,4-dinitrophenylhydrazone* which crystallized in yellow prisms from ethanol, m.p. 52–53 °C (Found : N, 9·7%. Calc. for $C_{30}H_{50}O_6N_4$ : N, 10·0%), and methylation with diazomethane gave the *methyl ester*, which crystallized in colourless needles, m.p. 76–77 °C from methanol (Found : C, 75·9 ; H, 12·3%. Calc. for $C_{25}H_{48}O_3$ : C, 75·7 ; H, 12·1%). These compounds showed no melting point depression when mixed with the synthetic specimens prepared below, and both specimens of the methyl ester had identical infra-red spectra, and had bands due to the carbonyl and the carbomethoxy groups.

(ii) *5-Ketodocosanoic Acid.*—Methyl 3-ketoeicosanoate was prepared by the method of Stallberg-Stenhagen and Stenhagen (1944). It was obtained as colourless needles, m.p. 54–55 °C (lit. m.p. 56·4 °C ; Found : C, 74·4 ; H, 11·8%. Calc. for $C_{21}H_{40}O_3$ : C, 74·1 ; H, 11·5%).

Ethyl β-bromopropionate (1·9 g) was refluxed with methyl 3-ketoeicosanoate (3·4 g) and dry potassium carbonate (5·0 g) in methyl *n*-propyl ketone (80 ml) for 20 hr. Water and ether were then added, the organic layer was washed with water and dried ($Na_2SO_4$), and the solvents removed by distillation. The residue, taken up in warm toluene (15 ml), was kept with a solution of potassium hydroxide (8 g) in water (8 ml) and methanol (110 ml) at 50 °C for 20 hr. The mixture was then acidified (dilute hydrochloric acid) and extracted with ether. The ether extracts were washed with water, dried, and evaporated. The residue on crystallization from ethanol gave 5-ketodocosanoic acid (2·0 g ; m.p. 92–93 °C). Stetter and Dierichs (1942) report m.p. 93–94 °C (Found : C, 74·8 ; H, 11·9%. Calc. for $C_{22}H_{42}O_3$ : C, 74·6 ; H, 11·8%).

(iii) *5-Ketotetracosanoic Acid.*—Methyl 3-ketodocosanoate was prepared according to the description of Marks and Polgar (1955). Alkylation with ethyl β-bromopropionate as described in Section V (*d*) (ii) gave 5-ketotetracosanoic acid. It was obtained as colourless prisms, m.p. 102–103 °C, from ethanol (Found : C, 75·6 ; H, 12·0%. Calc. for $C_{24}H_{46}O_3$ : C, 75·3 ; H, 12·1%). It gave a methyl ester, m.p. 76–77 °C, identical with that described above and on Wolff-Kishner reduction gave tetracosanoic acid, m.p. 83·5–84 °C, identified by comparison with an authentic specimen (Found : C, 78·4 ; H, 13·2%. Calc. for $C_{24}H_{48}O_2$ : C, 78·2 ; H, 13·1%).

*(e) Synthesis of 3-Alkylcyclohex-2-enones*

(i) *3-Heptadecylcyclohex-2-enone.*—Following the general method of Woods *et al.* (1949) a solution of 3-ethoxy*cyclo*hex-2-enone in dry ether was added to the Grignard reagent prepared from heptadecyl bromide. The crude ketone ($\lambda_{max.}$ 238 mμ ; ε 10,430), which was obtained in approximately 35–40% yield, was converted into its semicarbazone by treatment with a solution of semicarbazide acetate and sodium acetate in ethanol. The crude semicarbazone was purified by heating with light petroleum to remove *n*-tetratriacontane which is formed as a by-product in the reaction, and crystallized from ethanol or ethyl acetate. The *semicarbazone* was obtained as colourless prisms, m.p. 143–144 °C (Found : C, 74·0 ; H, 11·5 ; N, 10·9%. Calc. for $C_{24}H_{45}ON_3$ : C, 73·7 ; H, 11·5 ; N, 10·7%). The *2,4-dinitrophenylhydrazone* of 3-heptadecyl*cyclo*hex-2-enone crystallized from ethanol in orange prisms, m.p. 122–123 °C (Found : N, 10·5%. Calc. for $C_{29}H_{46}O_4N_4$ : N, 10·9%).

Catalytic hydrogenation of the crude unsaturated ketone in methyl acetate over platinum oxide gave crude 3-heptadecyl*cyclo*hexanone, which was converted into its semicarbazone. The purified *semicarbazone* crystallized as colourless prisms, m.p. 153–154 °C, from ethanol (Found : C, 73·2 ; H, 12·0 ; N, 10·6%. Calc. for $C_{24}H_{47}ON_3$ : C, 73·3 ; H, 11·9 ; N, 10·7%). Regeneration of the ketone from the semicarbazone by heating it in a solution of acetic acid with acetone containing sodium acetate gave *3-heptadecylcyclohexanone* which crystallized from ethanol in colourless needles, m.p. 48–49 °C (Found : C, 82·1 ; H, 13·0%. Calc. for $C_{23}H_{44}O$ : C, 82·1 ; H, 13·2%). The *2,4-dinitrophenylhydrazone* of 3-heptadecyl*cyclo*hexanone gave yellow prisms, m.p. 132–133·5 °C, from ethyl acetate (Found : N, 10·5%. Calc. for $C_{29}H_{48}O_4N_4$ : N, 10·8%).

(ii) *3-Nonadecylcyclohex-2-enone.*—Adding 3-ethoxy*cyclo*hex-2-enone to the Grignard reagent prepared from nonadecyl bromide gave crude 3-nonadecyl*cyclo*hex-2-enone which was also isolated as its semicarbazone, m.p. 141– 142 °C (Found : C, 74·8 ; H, 11·7 ; N, 9·6%. Calc. for $C_{26}H_{49}ON_3$ : C, 74·5 ; H, 11·7 ; N, 10·0%).

(iii) *3-Methylcyclohex-2-enone.*—Glutaryl chloride (12·6 g) was added to a solution of ethoxymagnesiomalonic ester in ether, prepared by the method of Adickes (1943) from magnesium (3·75 g). Distillation of the reaction product gave glutaryldimalonic ester (13·3 g) as a faintly yellow liquid, b.p. 224–225 °C/1 mm, which gave a strong reddish violet ferric test in ethanolic solution. This ester was previously prepared by Scheiber (1909) by an alternative method (Found : C, 55·4 ; H, 6·9%. Calc. for $C_{19}H_{28}O_{10}$ : C, 54·8 ; H, 6·7%). Hydrolysis of the ester by refluxing in aqueous sulphuric acid or in aqueous acetic acid-sulphuric acid mixture gave 3-methyl*cyclo*hex-2-enone in high yield. It was characterized as its semicarbazone, m.p. 200–201 °C (Found : C, 57·6 ; H, 7·8 ; N, 24·7%. Calc. for $C_8H_{13}ON_3$ : C, 57·5 ; H, 7·8 ; N, 25·1%) and 2,4-dinitrophenylhydrazone, m.p. 174–175 °C (Found : C, 53·9 ; H, 4·9 ; N, 19·1%. Calc. for $C_{13}H_{14}O_4N_4$ : C, 53·8 ; H, 4·8 ; N, 19·3%).

*(f) Phenols Derived from* C. auriculata *Oil*

The mixture of phenols (50 g) obtained by heating *C. auriculata* oil in ethanolic potassium hydroxide solution (Lamberton 1958*a*) was distilled at reduced pressure and the following fractions separated. Fraction (i), b.p. 230–232 °C/1 mm, comprised 40 g (80%) and was collected in several portions but these had identical physical properties and were combined. It was an almost colourless liquid which set to a solid crystalline mass at 0 °C and had $\lambda_{max.}$ 273, 279 mμ ; ε 1925, 1752 respectively (assuming mol. wt. 360). The infra-red spectrum showed a strong broad hydroxyl band at 3350 $cm^{-1}$ (liquid film) and no trace of carbonyl absorption. Fraction (ii), b.p. 233–240 °C/1 mm (*c.* 4·0 g) ; $\lambda_{max.}$ 273, 279 mμ ; ε 1760, 1654 respectively. Fraction (iii), b.p. 240–255 °C/1 mm (*c.* 4·0 g) ; $\lambda_{max.}$ 273, 279 mμ ; ε 1705, 1730 respectively.

When the oil used in the earlier investigation was distilled in this way 3-nonadecylphenol crystallized from the earlier fractions of the distillation. A specimen filtered from the oil and crystallized from light petroleum melted at 63–64 °C (Found : C, 83·3 ; H, 12·2%. Calc. for $C_{25}H_{44}O$ : C, 83·3 ; H, 12·2%).

A sample (7·0 g) of fraction (i) was completely methylated by heating with methyl iodide in acetone over anhydrous potassium carbonate for 8 hr. The total product was then hydroxylated

by the performic acid method using the experimental conditions described by Loev and Dawson (1956). After heating with alkali as described by these authors, the total product was chromatographed on neutral alumina. Light petroleum rapidly eluted 3-methoxynonadecylbenzene and the hydroxylated products remained adsorbed. The yield corresponded to 17% of 3-nonadecylphenol in the oil first examined, and 9–10% in the second specimen of oil.

Elution of the more strongly adsorbed hydroxylated compounds and crystallization from light petroleum gave colourless needles (5·5–6·0 g) with an indefinite melting point, about 38–40 °C (Found : C, 76·7 ; H, 11·6%. Calc. for $C_{26}H_{46}O_2$ (corresponding to a glycol from the methyl ether of a nonadecenyl phenol) : C, 76·8 ; H, 11·3%). Periodate oxidation gave a mixture of short-chain aldehydes which were converted into their 2,4-dinitrophenylhydrazones, but these were not separated.

### (*g*) *Long-Chain Quinols and Quinones*

The higher boiling phenolic fractions (ii) and (iii) obtained from the second sample of *C. auriculata* oil (Section V (*f*)) had a somewhat different ultraviolet absorption from fraction (i) ; the maximum at λ 273 mμ was less intense and there was more absorption in the λ 290 mμ region. On standing, crystalline material separated from both fractions and this was easily isolated because of its sparing solubility in light petroleum. Further quantities of material insoluble in light petroleum were obtained by hydrogenating each fraction and again crystallizing from light petroleum. The total yield amounted to about 2–3% of the original oil.

When these fractions were combined and crystallized several times from light petroleum this material was obtained as colourless prisms, m.p. 69–70 °C (with shrinking from around 65 °C), which had $\lambda_{max.}$ 292 mμ ; ε 3550 (assuming mol. wt. 376), and the infra-red spectrum showed only hydroxyl absorption (Found : C, 80·4 ; H, 11·2%. For comparison nonadecyl quinol requires C, 79·9 ; H, 11·7%). When this material was crystallized from aqueous ethanol a deep blue colour developed as the solution cooled. Oxidation by shaking with silver oxide in dry ether according to the method of Cook, Heilbron, and Lewis (1942) gave a yellow mixture of quinones which were chromatographed on alumina. Elution by light petroleum and crystallization from ethanol gave pale yellow needles, m.p. 58–60 °C, with shrinking from *c.* 55 °C ; $\lambda_{max.}$ 247 mμ ; ε 15,420 (in ethanol) (Found : C, 80·7 ; H, 10·8%. For comparison, calc. for nonadecyl benzoquinone : C, 80·2 ; H, 11·3%).

These mixtures were not easily separated into their components but when the quinol was isolated from fraction (ii) alone by hydrogenating it and separating the portion insoluble in light petroleum, oxidation and purification of the resulting quinone gave pale yellow needles, m.p. 80–81 °C, which were identical with a synthetic specimen of nonadecylbenzoquinone (Found : C, 80·3 ; H, 11·2%. Calc. for $C_{25}H_{42}O_2$ : C, 80·2 ; H, 11·3%).

A similar mixture of long-chain quinones was also obtained by silver oxide oxidation of each of the following, in each case after hydrogenation and crystallization from light petroleum :

(i) the last fraction (5%) obtained in the distillation of *C. auriculata* oil ;
(ii) the last fraction (5%) in the distillation of *C. auriculata* oil, which has been previously heated with ethanolic hydrochloric acid ;
(iii) the last fraction (5%) in the distillation of hydrogenated *C. auriculata* oil.

### (*h*) *Synthesis of Long-Chain Quinones*

A Friedel-Craft reaction between nonadecanoyl chloride and hydroquinone dimethyl ether under the conditions described by Cook, Heilbron, and Lewis (1942) gave *2,5-dimethoxyphenyl octadecyl ketone* which crystallized in colourless prisms, m.p. 55–56 °C, from methanol (Found : C, 77·8 ; H, 11·1%. Calc. for $C_{27}H_{46}O_3$ : C, 77·5 ; H, 11·0%). It was characterized by the formation of a *2,4-dinitrophenylhydrazone*, red prisms, m.p. 81–82 °C, from ethanol (Found : N, 9·1%. Calc. for $C_{33}H_{50}O_6N_4$ : N, 9·3%), and by a strong carbonyl band (1682 $cm^{-1}$) in the infra-red spectrum. Under similar conditions heptadecanoyl chloride gave *2,5-dimethoxyphenyl hexadecyl ketone*, which crystallized from methanol in colourless prisms, m.p. 45–46 °C, infra-red carbonyl band at 1682 $cm^{-1}$ (Found : C, 77·2 ; H, 10·8%. Calc. for $C_{25}H_{42}O_3$ : C, 76·9 ; H, 10·8%). 2,5-Dimethoxyphenyl heptadecyl ketone (i.e. 2,5-dimethoxystearophenone) pre-

pared from stearoyl chloride had m.p. 56–56·5 °C and the infra-red carbonyl band at 1682 $cm^{-1}$. Cook, Heilbron, and Lewis report m.p. 46 °C for this compound but this is probably a typographical error.

Wolff-Kishner reduction (Cook, Heilbron, and Lewis (1942) used a Clemmensen reduction for this step) of the acylquinol ethers in diethylene glycol gave the corresponding alkylquinol ethers. *2,5-Dimethoxynonadecylbenzene* was obtained as colourless prisms, m.p. 54–55 °C, from ethanol and showed no trace of carbonyl or hydroxyl absorption in the infra-red spectrum (Found : C, 80·3 ; H, 11·9%. Calc. for $C_{27}H_{48}O_2$ : C, 80·1 ; H, 12·0%). *2,5-Dimethoxyheptadecylbenzene*, m.p. 47–48 °C (Found : C, 79·8 ; H, 11·8%. Calc. for $C_{25}H_{44}O_2$ : C, 79·8 ; H, 11·7%). 2,5-Dimethoxyoctadecylbenzene, m.p. 52–53 °C, was previously described by Cook, Heilbron, and Lewis (1942), who report its boiling point but not its melting point.

Demethylation of the 2,5-dimethoxyalkylbenzenes by heating with acetic acid-hydrobromic acid and oxidation of the resulting crude quinols with silver oxide in ether gave the alkylbenzoquinones, which were purified by chromatography on alumina and crystallized from ethanol. Nonadecylbenzoquinone, pale yellow needles, m.p. 80–81 °C, was found to be identical with the quinone isolated above (Found : C, 79·9 ; H, 11·3%. Calc. for $C_{25}H_{42}O_2$ : C, 80·2 ; H, 11·3%). *Heptadecylbenzoquinone* was obtained as pale yellow needles, m.p. 72–73 °C (Found : C, 79·6 ; H, 11·0%. Calc. for $C_{23}H_{38}O_2$ : C, 79·8 ; H, 11·0%).

An attempt to prepare the quinones directly from the 2,5-dimethoxyalkylbenzenes by oxidative demethylation with nitric acid was unsuccessful because nitration also took place.

### (*i*) *The Exudate from* C. macrophylla *Hook.f.*

A specimen of exudate from *C. macrophylla* consisted of a slightly milky aqueous emulsion (*c*, 500 ml) and extraction with ether gave very little oil (approx. 1 g). The oil had $[\alpha]_D$ −8° (*c*, 3·5 in ethanol) and no characteristic ultraviolet absorption maxima. The infra-red spectrum (liquid film) showed a strong band at 3400 $cm^{-1}$ and minor peaks at 1645, 1670, and 1710 $cm^{-1}$.

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# STUDIES OF THE OPTICALLY ACTIVE COMPOUNDS OF ANACARDIACEAE EXUDATES

## VI. THE EXUDATE FROM PENTASPADON OFFICINALIS HOLMES

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*Summary*

The oil from the wood of *Pentaspadon officinalis* Holmes (family Anacardiaceae) consists largely of 2-hydroxy-6-(heptadeca-*cis*-8′,*cis*,-11′-dienyl)benzoic acid. It is very similar to the oil from *P. motleyi* Hook.f. and does not contain optically active *cyclo*hexenones of the type found in *Campnosperma* exudates.

### I. INTRODUCTION

In previous papers of this series it was shown that the exudates from *Campnosperma brevipetiolata* and *C. auriculata* contain optically active long-chain *cyclo*hexenones, and it seemed desirable to know whether compounds of this type were present in the oily exudates from the wood of other trees of the family Anacardiaceae. Earlier investigations of the oil from *Pentaspadon motleyi* Hook.f. by van Romburgh and van Veen (1929); van Romburgh, van Veen, and Haagen-Smit (1930), and finally by Backer and Haack (1941) led to the formulation of pelandjauic acid, the principal component, as 2-hydroxy-6-heptadecadienylbenzoic acid. Backer and Haack showed that reduction of the oil gives 2-hydroxy-6-heptadecylbenzoic acid (I), and that decarboxylation followed by reduction gives 3-heptadecylphenol, which they synthesized. Jones and Smith (1929), from a similar oil from a New Guinea tree identified as " near *Pentaspadon motleyi* ", obtained pentaspadonic acid which from the description of its reduction and other reaction products appears to be pelandjauic acid.

Although the experimental results in these investigations of the oil from *P. motleyi* are in substantial agreement, it is not clear whether any non-aromatic compounds like those of the *Campnosperma* exudates are initially present in the oil, before it is subjected to chemical treatment or distillation which would cause their decomposition. It has not been possible to obtain a specimen of the oil from *P. motleyi* to check this point, but an analysis has now been made of the oil from *P. officinalis* Holmes, a Malayan tree of the same genus.

### II. ANALYSIS OF THE OIL

The light brown oil from *P. officinalis* gives an intense violet colour with ferric chloride in alcoholic solution, and on catalytic hydrogenation it gives an almost quantitative yield of 2-hydroxy-6-heptadecylbenzoic acid (I). The absence of *cyclo*hexenones like those found in *Campnosperma* exudates is shown

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by the lack of optical activity of the oil, the high yield of I on hydrogenation, and by the almost complete identity of the ultraviolet absorption of the oil (shown in Fig. 1) with that of I. Decarboxylation of I by distillation under reduced pressure gives a high yield of 3-heptadecylphenol, and similarly distillation of the original oil gives unsaturated phenols ; again both the saturated and unsaturated phenols have virtually identical ultraviolet absorption.

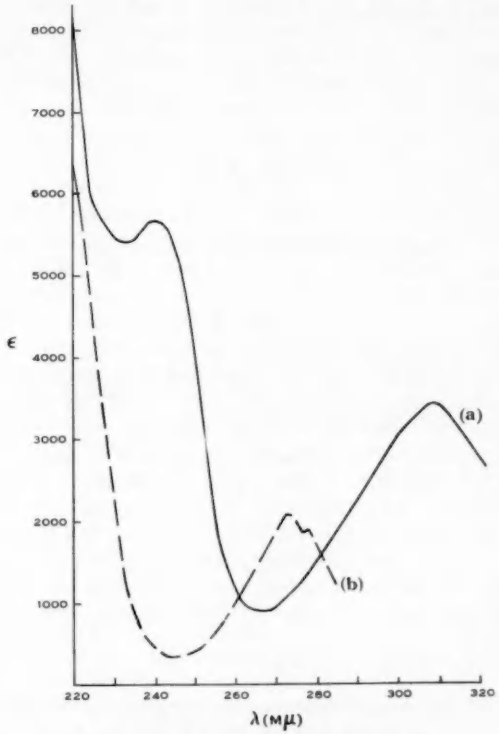


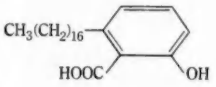

Fig. 1.—Ultraviolet absorption spectra.

(*a*) Oil from *Pentaspadon officinalis*, in ethanol.

(*b*) Unsaturated phenol, from distillation of *Pentaspadon officinalis* oil, in ethanol.

The uptake of hydrogen during the catalytic reduction is equivalent to about 1·9 double bonds per molecule, and it is evident from the ultraviolet absorption of the saturated and unsaturated material that the side chain contains no double bonds in conjugation. When the unsaturated phenols obtained by distillation of the oil are heated with potassium hydroxide in ethylene glycol at 180 °C, the ultraviolet absorption of the recovered phenols then shows an intense maximum at λ 227 mμ, and if a correction is made for the absorption contributed

by the hydroxyphenyl chromophore, the resulting ultraviolet spectrum shows a maximum at λ 232 mμ ; ε 17,700. This change in the ultraviolet absorption is characteristic of the "pentadiene", or allylic system II, and similar isomerizations of fatty acids have been discussed by Pitt and Morton (1957). The presence of bands at 947 and 982 $cm^{-1}$ in the infra-red spectrum of the isomerized unsaturated phenols indicates the presence of *trans*-double bonds in conjugation ; the alkali isomerization evidently involves a change in the geometrical configuration as well as the position of the bonds.

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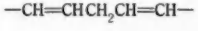


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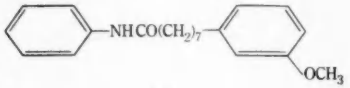


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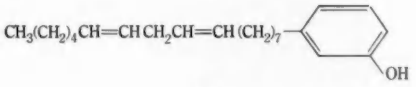


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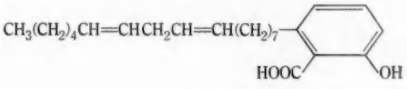


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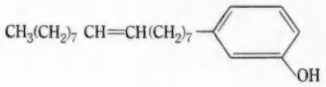


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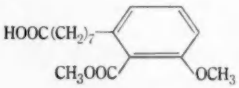


(VII)

Ozonolysis of the methyl ethers of the unsaturated phenols gives a high yield of a mixture of steam-volatile short-chain aldehydes, consisting predominantly of *n*-hexaldehyde, which is easily separated as its 2,4-dinitrophenylhydrazone. Moreover, oxidation of the non-volatile ozonolysis products with potassium permanganate yields an acid which is converted into an anilide having analytical values in agreement with the formula $C_{21}H_{27}O_2N$, and is consequently the anilide of ω-(3-methoxyphenyl)octanoic acid (III). This leads to the formula IV for the principal component of the unsaturated phenols, and consequently to V for the principal component of the original oil. The absence of the bands at about 960 $cm^{-1}$, characteristic of a *trans*-bond, from the infra-red spectra of IV and of its methyl ether, shows that IV has the *cis-cis-* configuration. In agreement with this the rate of isomerization of *cis-trans-* and *trans-trans-*compounds to a conjugated system is generally slower than that observed.

Hydroxylation of the methyl ethers from the unsaturated phenols by the performic acid method gives a mixture of products which is very difficult to separate. A poor yield of hydroxylated products from the methyl ether of IV is to be expected for it has been shown (McKay, Levitin, and Jones 1954) that the peracid oxidation of linoleic acid is abnormal, giving compounds having a cyclic ether ring, and only poor yields of tetrahydroxy acids. From the periodate oxidation of the crude mixture of hydroxylation products only a low yield of a mixture of short-chain aldehydes is obtained, and fractional crystallization of their 2,4-dinitrophenylhydrazones finally gives pure nonaldehyde 2,4-dinitrophenylhydrazone. The formation of a high proportion of nonaldehyde in the oxidation products by this method is most satisfactorily explained by assuming the presence of a monoethylenic phenol (VI) in the unsaturated phenolic oil. Nonaldehyde would be formed in high yield from oxidation of the methyl ether of VI whereas hexaldehyde could only be expected in poor yield from the methyl ether of IV, and consequently the proportion of nonaldehyde is much higher than in the ozonolysis products. Like IV, the monoethylenic phenol VI must be produced by decarboxylation of the corresponding acid in the distillation of the original oil. Oxidation of the methyl ethers of both IV and VI leads to ω-(3-methoxyphenyl)octanoic acid, and this agrees with the isolation of the uniform product III from the ozonolysis.

Backer and Haack (1941) did not investigate the nature of the heptadecadienyl chain in pelandjauic acid, but Jones and Smith (1929) reported the isolation of hexanoic acid and the acid VII from the oxidation of their methylated pentaspadonic acid. The latter authors assigned a structure having a $n$-$C_{16}$ chain and two double bonds in conjugation, but taking the chain length as $C_{17}$, as established by Backer and Haack, these oxidation products are also consistent with the formula V. The oils from *P. officinalis* and *P. motleyi* are thus very closely similar, and it is of biochemical interest that the major component of each apparently contains a heptadecadienyl chain as in linoleic acid. In view of the close similarity of the oils it may be noted that Burkhill (1935) describes *P. officinalis* Holmes as scarcely distinct from *P. motleyi* Hook.f.

## III. Experimental

### (a) *General*

As in Part I of this series (Dalton and Lamberton 1958).

### (b) *Oil from* P. officinalis

As it was obtained the exudate consisted of a partly emulsified oil in aqueous suspension. The oil was extracted with light petroleum, the extracts dried over sodium sulphate, and the solvent evaporated under reduced pressure. The residue was a light brown mobile oil, optically inactive, which gave an intense violet colour with ferric chloride in ethanol. Ultraviolet absorption: $\lambda_{max.}$ 242, 309 mμ; ε 5890, 3420 respectively.

Distillation at 1 mm pressure gave the unsaturated phenol III as a colourless oil, b.p. 224–226 °C, which did not give a colour with ferric chloride, and had $\lambda_{max.}$ 273, 278 mμ; ε 2095, 1830 respectively. Yield, 75–80%.

### (c) *2-Hydroxy-6-heptadecylbenzoic Acid* (*I*)

The oil (3·0 g) dissolved in methyl acetate was hydrogenated over platinum oxide until uptake of hydrogen ceased. Evaporation of the solvent left a crystalline residue which gave I

as long needles, m.p. 93–94 °C, on crystallization from light petroleum (boiling range 60–80 °C). Van Romburgh, van Veen, and Haagen-Smit (1930) report m.p. 93–94 °C. The hydrogen absorption was approximately equivalent to two double bonds per molecule, and the yield of crystalline product was 80–85% with further amounts remaining in the mother liquors. The purified acid gave a strong violet colour with ferric chloride in ethanol, and had $\lambda_{max.}$ 242, 309 mμ; ε 5480, 3480 respectively (Found: C, 76·7; H, 10·6%. Calc. for $C_{24}H_{40}O_3$: C, 76·6; H, 10·6%). The *acetate* of I crystallized in colourless needles, m.p. 81–82 °C, from light petroleum (Found: C, 74·8; H, 10·0%. Calc. for $C_{26}H_{42}O_4$: C, 74·6; H, 10·0%).

Distillation of the acid I at 1 mm pressure gave 3-heptadecylphenol in 75–80% yield. It had $\lambda_{max.}$ 273, 278 mμ; ε 1970, 1820 respectively, m.p. 59–60 °C, and gave no colour with ferric chloride in ethanol. Backer and Haack (1941) report m.p. 59·5–60 °C (Found: C, 83·2; H, 12·1%. Calc. for $C_{23}H_{40}O$: C, 83·1; H, 12·0%). The methyl ether melted at 37·5–38 °C. Backer and Haack report m.p. 37·5–38 °C.

### (*d*) *Isomerization of the Unsaturated Phenol* (*III*)

The method is based on the procedure of Mitchell, Kraybill, and Zscheile (1943). The unsaturated phenol (0·5 g) was added to a solution of potassium hydroxide (3·75 g) in ethylene glycol (50 ml) and the mixture heated at 180 °C for 25 min. After dilution with water and acidification, the phenol was extracted with light petroleum and distilled at 1 mm pressure. This gave a colourless oil having $\lambda_{max.}$ 227, 268 mμ; ε 25,500, 4200 respectively. The isomerized phenol had an infra-red spectrum with a strong hydroxyl band (3350 $cm^{-1}$) which resembled the spectrum of 3-nonadecylphenol, but it also had two medium sharp bands at 947 and 982 $cm^{-1}$, indicating *trans*-bonds in conjugation.

### (*e*) *Oxidation Products*

The methyl ethers of the unsaturated phenols were prepared by heating a solution of the unsaturated phenols and methyl iodide in dry acetone over potassium carbonate. The methyl ethers were obtained as a colourless liquid, b.p. 218–220 °C at 1 mm. Their infra-red spectrum showed the absence of hydroxyl groups and closely resembled that of 3-methoxynonadecylbenzene.

(i) *Ozonolysis.*—The unsaturated methyl ethers (2·0 g) dissolved in methyl acetate were ozonized at 0 °C, and after catalytic hydrogenation of the ozonides, the solvent was distilled. Steam distillation of the residue gave a volatile oil which was converted into a mixture of 2,4-dinitrophenylhydrazones. Several crystallizations from ethanol gave *n*-hexaldehyde 2,4-dinitrophenylhydrazone, m.p. 104–105 °C. Its melting point was undepressed by mixture with an authentic specimen, but a mixture with nonaldehyde 2,4-dinitrophenylhydrazone melted at 88–90 °C (Found: C, 51·2; H, 5·7; N, 20·1%. Calc. for $C_{12}H_{16}O_4N_4$: C, 51·4; H, 5·7; N, 20·0%). The ozonolysis products which were not steam volatile were extracted with ether and oxidized with potassium permanganate in acetone. Distillation of the acidic product gave a colourless oil which slowly crystallized on standing. Conversion into its acyl chloride by heating with oxalyl chloride, followed by addition of aniline, gave an *anilide*, which crystallized in colourless needles, m.p. 66–67 °C, from slightly aqueous ethanol (Found: C, 77·7; H, 8·4; N, 4·5; $OCH_3$: 9·4%. Calc. for $C_{21}H_{27}O_2N$: C, 77·5; H, 8·3; N, 4·3; $OCH_3$: 9·5% (one methoxyl)).

(ii) *Peracid Oxidation.*—Hydroxylation of the unsaturated methyl ethers by the performic acid method (following the procedure described by Loev and Dawson 1956), and heating with alkali gave an oily mixture which was not separated but was immediately oxidized with potassium periodate and the volatile aldehydes isolated from the reaction products by steam distillation. Fractional crystallization of the mixture of 2,4-dinitrophenylhydrazones prepared from these aldehydes finally gave yellow needles, m.p. 104–105 °C. Mixed with the 2,4-dinitrophenylhydrazones of *n*-hexaldehyde and *n*-heptaldehyde these melted at 84–87 and 88–90 °C respectively, but the melting point was not depressed by mixing with the 2,4-dinitrophenylhydrazone of nonaldehyde (Found: C, 56·0; H, 7·1; N, 17·1%. Calc. for $C_{15}H_{22}O_4N_4$: C, 55·9; H, 6·8; N, 17·4%).

## IV. Acknowledgments

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# STUDIES OF A QUINYL-*p*-COUMARATE IN THE PINEAPPLE PLANT (*ANANAS COMOSUS* VAR. CAYENNE)*

By G. K. SUTHERLAND† and W. A. GORTNER‡

[*Manuscript received November* 17, 1958]

*Summary*

An ester is found in small concentrations in vegetative pineapple plants, with spectral characteristics in the ultraviolet of an ester of *p*-coumaric acid. *p*-Coumaric acid is obtained after hydrolysis, and the remaining aqueous hydrolysate indicates the presence of quinic acid lactone on chromatograms. On the basis of neutral equivalent determinations, boric acid conductivity and periodate oxidation experiments, and analyses following mild hydrolysis, the structure of the ester is suggested to be a quinyl-di-*p*-coumarate. It serves in the plant as a cofactor for pineapple indoleacetic acid oxidase.

## I. INTRODUCTION

The rather widespread occurrence of hydroxycinnamic acids and esters has only recently been recognized (Bate-Smith 1956). The presence of these acids in hydrolysed leaf extracts has been tabulated by the same author (Bate-Smith 1954). Apart from the geometric isomerism which can occur because of the double bond in cinnamic acids, and which has been reported to show up on chromatograms (Williams 1955), esterification with quinic acid leads to a variety of position isomers. Caffeic acid is known to occur frequently as an ester with quinic acid (chlorogenic acid, I) in leaves and fruit (Bradfield *et al.* 1952). *iso*Chlorogenic acid has been reported in coffee, and a structure was assigned (Barnes, Feldman, and White 1950) on the basis of conductivity and periodate-oxidation experiments. Other isomers have been indicated but no structure determinations made, e.g. *neo*chlorogenic acid from peaches (Corse 1953) and *pseudo*chlorogenic acid from sweet potatoes (Uritani and Miyano 1955). From artichoke, *Cynara scolymus*, a quinyldicaffeate (Panizzi and Scarpati 1954*a*) has been isolated and identified in which caffeic acid groups are esterified through the hydroxyl groups on carbon atoms numbers 1 and 4 of the quinic acid ring. The suspected presence of trace amounts of *p*-coumarylquinic acids was reported in tea leaf (Cartwright *et al.* 1955), and similar substances in both leaf and fruit of the apple and pear. These compounds were found to give chromatographic

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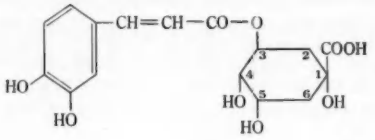
evidence for *p*-coumaric acid (II, R=H) and quinic acid after acid hydrolysis, but no suggestions for the possible structures of the esters have been advanced.

The present paper reports on the occurrence of a biologically-active quinyl ester of *p*-coumaric acid in pineapple stem tissue. The *p*-coumaric acid ester is one of two naturally occurring polyphenols acting as modifiers of pineapple indoleacetic acid oxidase (Gortner and Kent 1958 ; Gortner, Kent, and Sutherland 1958).

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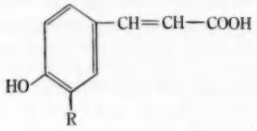


(II)

## II. Experimental

(*a*) *Concentration of the Ester.*—The leaves and roots were removed from vegetative pineapple plants, and the stem tissue was extracted by blending with 1·4 times its weight of water. After partial clarification in a basket centrifuge, the suspension was heated to 90–95 °C for a short time, then filtered with the aid of " Dicalite ". Hydrochloric acid was added to lower the pH to 1·6–1·7 and the solution was extracted twice with ethyl acetate, using centrifugation to break the stable emulsion which formed. Removal from the ethyl acetate into saturated sodium bicarbonate was followed by acidification and extraction into ether. This solution was stored in the cold until required. An aqueous solution was prepared by evaporating the ether from a water-ether mixture stirred by a magnetic stirrer. This solution was lyophilized and the resulting yellow-white solid transferred to a sample bottle in a low humidity atmosphere, and stored in a desiccator. This removal into aqueous solution and lyophilization apparently caused some hydrolysis of the ester, since free quinic and *p*-coumaric acids appeared as new spots on subsequent chromatograms. The ester could also be prepared from tissue extracts by precipitation with lead acetate, regeneration with sulphuric acid, and extraction into ether or ethyl acetate. The resulting ester preparation was comparable in purity to that prepared by solvent partition as described.

(*b*) *Absorption Measurements.*—All ultraviolet absorption spectra were determined over the range 210–400 mμ on a Beckmann Model DU Spectrophotometer at concentrations approximately $5\times10^{-5}$M, using 1 cm silica cells. Infra-red measurements were made on a " Nujol mull " of the sample in a Beckmann Model IR–2A Spectrophotometer.

(*c*) *Preparation of Derivatives.*—Methylation, acetylation, and reduction were carried out after the methods of Panizzi and Scarpati (1954*b*). Hydrolyses were performed by boiling the extract for several minutes with 2N hydrochloric acid or 1N sodium hydroxide. Dissolving the extract in 1N sodium hydroxide for 6 hr at room temperature under an atmosphere of nitrogen showed spectral evidence for complete hydrolysis. Ether extraction after acidification permitted recovery of *p*-coumaric acid, while desalting the aqueous residue on a " Dowex 50–X " column yielded quinide, m.p. 188–192 °C (uncorr.).

Bromination of the extract was attempted by adding an excess of bromine water to a warm (60–65 °C) aqueous solution of the extract. The precipitate which formed was recrystallized from aqueous ethanol, m.p. 93·5–96 °C (uncorr.) (Found (average of duplicates) : C, 22·3 ; H, 1·1 ; O, 5·0 ; Br, 71·6%. Calc. for tribromophenol, $C_6H_3OBr_3$ : C, 21·8 ; H, 0·9 ; O, 4·8 ; Br, 72·5%).

(*d*) *Neutral Equivalent Determinations.*—These were carried out in aqueous ethanol using 0·1N aqueous sodium hydroxide, or in *NN*-dimethylformamide using 0·1N sodium methoxide solution in methanol/benzene (Fritz 1952). A Beckmann Model G ph meter was used equipped with glass and calomel electrodes and a magnetic stirrer.

(*e*) *Conductivity Experiments.*—Resistance measurements were made on a Conductivity Bridge (Industrial Instruments Co., Model 16–B–1) in a 25 °C constant temperature bath using 0·1M boric acid solutions and $10^{-3}$M solutions of the material in question. The conductivity cell had a cell constant of 0·4311.

(*f*) *Mole Ratio Determinations.*—After alkaline hydrolysis at room temperature for 6 hr and subsequent acidification, the sample was evaporated and dissolved in ethanol. The *p*-coumaric acid concentration was measured spectrophotometrically using the absorbance at 312 mμ, log $\varepsilon_{312}=4\cdot36$ (Jurd 1956). Quinic acid was determined by oxidation for 10 min at room temperature with periodic acid, followed by an iodometric titration (Siggia 1949).

(*g*) *Synthesis of Control Compounds.*—*p*-Coumaric acid was prepared according to the method of Pandya and Vahidy (1936), and recrystallized from hot water, m.p. 210·5–213·5 °C. *p*-Methoxy cinnamic acid was prepared by a similar reaction ; it melted to an opalescent liquid by 172 °C and changed to a clear liquid at 187 °C (Skau and Meier 1935). Quinide was synthesized from quinic acid (Panizzi and Scarpati 1954c), m.p. 193–195 °C.

## III. Results and Discussion

### (*a*) *Identification as a* p-*Coumaric Ester*

The only method found suitable for preparing a solid extract from pineapple plants was to lyophilize a partially purified aqueous solution. The yellowish white material so obtained was very unstable and became brown and resinous after a short exposure to the atmosphere. When dissolved in water or ethanol, the solid gave an ultraviolet spectrum almost identical with that of *p*-coumaric acid (Sutherland 1958). In ethanolic sodium ethoxide or sodium hydroxide, a strong bathochromic shift was observed and the solution became yellow (Table 1).

Table 1

ULTRAVIOLET ABSORPTION DATA

| Compound | EtOH | | NaOEt in EtOH | |
|---|---|---|---|---|
| | $\lambda_{max.}$ (mμ) | $\lambda_{min.}$ (mμ) | $\lambda_{max.}$ (mμ) | $\lambda_{min.}$ (mμ) |
| *p*-Coumaric acid .. .. .. | 312 | 248 | 337 | 260 |
| Extract .. .. .. .. .. | 314 | 254 | 365 | 272 |
| Hydrolysed extract .. .. .. | 312 | 253 | 337 | 261 |
| Reduced, hydrolysed extract .. .. | 280* | 250 | 282* | |
| Acetylated, hydrolysed extract .. | 285 | | | |
| Methylated, hydrolysed extract .. | 305 | 251 | 280 | 247 |
| *p*-Methoxycinnamic acid .. .. | 305 | 247 | 283 | 243 |

* Very pronounced decrease in absorbance.

The shape of this alkaline spectral curve was almost identical with that of methyl *p*-coumarate in the same solvents.

The ultraviolet absorption curve in 1N sodium hydroxide altered slowly over a 6-hr period and resulted in a new spectrum identical with that of *p*-coumaric acid in this solvent ($\lambda_{max.}$ 337, $\lambda_{min.}$ 261, inflection point 315 mμ). After reacidification, the spectral curve was the same shape as before the alkaline treatment, but was displaced slightly to lower wavelengths, indicating the release of *p*-coumaric acid from the ester by alkaline hydrolysis.

The usual reactions to prepare derivatives were attempted without the isolation of any crystalline materials. The reactions attempted included partial and complete methylation, reduction, and acetylation. That these reactions did in fact proceed could be demonstrated by chromatography and by observing the changes in spectral absorption (Table 1); indeed, they thus serve as additional indications of structure. Catalytic hydrogenation resulted in reduction of the double bond conjugated to the benzene ring; the spectrum of the derivative after hydrolysis was identical with that of *p*-hydroxyhydrocinnamic acid. Methylation of the phenolic group also could be demonstrated through spectrum changes, with *p*-methoxycinnamic acid being released upon hydrolysing the methylated extract, as evidenced by $R_F$ on paper chromatograms, by reaction to spray reagents, and by absorption spectrum.

TABLE 2

$R_F$ VALUES IN VARIOUS SOLVENT SYSTEMS*

| Solvent | Ester A | Ester A Hydrolysed | *p*-Coumaric Acid | Ester B |
|---|---|---|---|---|
| *n*-Butanol/acetic acid/water (4 : 1 : 5) | 0·86 | 0·92 | 0·92 | 0·82 |
| Phenol/water (73 : 27 w/v) .. .. | 0·30 | 0·80 | 0·84 | 0·40 |
| Ethyl acetate/conc. ammonia (1 : 1) | 0 | 0·07 | 0 | 0 |
| Ethyl acetate/conc. HCl (1 : 1) .. | —† | 0·87 | 0·87 | —† |
| Toluene/acetic acid/water (4 : 1 : 5) .. | 0 | 0·09 | 0·10 | 0 |
| Acetic acid/conc. HCl/water (30 : 3 : 10) | 0·89 | | 0·88 | 0·92 |

* Ascending chromatography on Whatman No. 1 paper.

† Pronounced streaking.

Bromination of the extract in warm aqueous solution resulted in the formation in low yield of 2,4,6-tribromophenol. This compound was identified by melting point, elemental analyses, and by comparison of ultraviolet and infra-red spectra with an authentic sample. Tribromophenol was also obtained from a similar bromination of *p*-coumaric acid, but no similar bromine derivative was obtained from caffeic or chlorogenic acids. Displacement of the cinnamic side chain by bromination of *p*-coumaric acid does not appear to have been reported previously, but is not completely unexpected in view of the displacement of COOH, $HSO_3$, CHO, and $CH_2OH$ groups *ortho* or *para* to a phenolic group by bromination reported by other investigators (Ruderman 1946). The bromination of *p*-coumaric acid in glacial acetic acid rather than in water has been reported (Zincke and Leisse 1902) to give αβ-dibromo-β-(3,5-dibromo-4-hydroxyphenyl)-propionic acid; no material was recovered when our extract was treated with bromine under these conditions.

When the extract was chromatographed on paper in a variety of solvents, two generally overlapping spots were seen under ultraviolet light. Two-dimensional chromatography failed to show any new spots. $R_F$ values in various solvents are presented in Table 2. Spot A fluoresced dark blue, while spot B was light blue. It could be shown that spot A was produced by the material

responsible for the ultraviolet spectrum in ethanol by taking the spectrum of the material directly on the paper, after the method of Bradfield and Flood (1952). The major component, ester spot A, was also located by spraying chromatograms with diazotized *p*-nitroaniline (Swain 1953), when a red-brown colour was formed.

After hydrolysis two new spots showing similar fluorescence as before appeared on chromatograms. These spots agreed in $R_F$ values and in colours developed by spray reagents with data found (Swain 1953; Bate-Smith 1956) for *p*-coumaric acid and ferulic acid (II, R=$OCH_3$), of which the former acid was present in much larger amounts. Large-scale extraction and subsequent acid hydrolysis allowed recovery of *p*-coumaric acid, which was shown to be contaminated with ferulic acid by chromatography in toluene–acetic acid–water (Bate-Smith 1956). The infra-red spectrum of this derivative was almost identical with that of authentic *p*-coumaric acid. All attempts to separate the *p*-coumaric acid from the accompanying ferulic acid were unsuccessful. The evidence outlined above indicated the presence of a *p*-coumaric acid ester in the plant, but this component could not be freed from the ferulic acid ester which appeared to be present by the usual techniques of adsorption chromatography, partition chromatography, or ion exchange.

### (*b*) *Identification of the Quinic Acid Fragment*

After acid hydrolysis and removal of the *p*-coumaric acid by ether extraction, the aqueous residue was chromatographed on paper and showed a periodate-reactive spot (Cifonelli and Smith 1954; Cartwright *et al.* 1955) identical with the similar fragment from acid-hydrolysed chlorogenic acid. From the recorded $R_F$ values in various solvents this spot was shown to be due to quinide, the γ-lactone of quinic acid. Small colourless crystals of quinide were obtained from the aqueous fraction after alkaline hydrolysis and ether extraction, and were identified by melting point and chromatography with synthetic quinide.

None of the derivative-forming reactions which served to characterize the *p*-coumaric acid moiety changed the chromatographic characteristics of the quinic acid fragment after hydrolysis.

### (*c*) *Structural Data*

Mild alkaline hydrolysis followed by estimation of the resulting concentrations of *p*-coumaric acid and quinic acid gave variable results but approximated a *p*-coumaric/quinic ratio of 2 : 1. Under the conditions of the hydrolysis, *p*-coumaric acid was shown to be stable over a 24-hr period. Neutral equivalent determinations run on the extract in *NN*-dimethylformamide yielded no quantitative data, since the titration curve showed an immediate sharp rise. Quinide showed a similarly shaped titration curve in this solvent, although chlorogenic acid* gave a reliable titre. This was interpreted as indicating a free-hydroxyl group attached to carbon 3 of the quinic acid part of the molecule, which could result in lactone formation under the dehydrating conditions in the preparation of the lyophilized sample. This was substantiated by conductivity data on the extract and related compounds in water and in the presence of boric acid (Table 3).

* Kindly furnished by Central Laboratories, General Foods Corporation, New Jersey.

Although the literature on conductivity alterations due to the presence of boric acid is somewhat confused, the availability of vicinal *cis*-hydroxyl groups seems to be essential for positive results with *cyclo*hexane derivatives (Böeseken 1949). The data thus indicated that one or both of the hydroxyl groups at carbons 4 and 5 of the quinic acid fragment must be involved in the esterification. The absence of periodate activity of the extract, after purification by chromatography in *n*-butanol–acetic acid–water (4 : 1 : 2·2) and using the glycol-reactive spray (Cifonelli and Smith 1954), showed an attachment must exist through carbon 4.

TABLE 3

EFFECT OF BORIC ACID ON CONDUCTANCE OF QUINIC ACID COMPOUNDS ($1 \times 10^{-3}$M)

| Compound | $K$* Sample (ohms$^{-1}$) | $K$ Sample and Boric Acid (ohms$^{-1}$) | $\Delta K$ ($\times 10^6$) |
|---|---|---|---|
| Chlorogenic acid .. .. | $1{\cdot}31 \times 10^{-4}$ | $1{\cdot}98 \times 10^{-4}$ | 67 |
| Quinic acid .. .. .. | 1·35 | 1·81 | 46 |
| Caffeic acid .. .. .. | 0·50 | 0·55 | 5 |
| *p*-Coumaric acid .. .. | 0·60 | 0·59 | —1 |
| Extract .. .. .. | 1·23 | 1·21 | —2 |

* Specific conductance $K$ = cell constant/resistance.

The data on structure of the main ester component are compatible with quinyl-1,4-di-*p*-coumarate, but final proof of this structure must await further investigation.

Assays indicate that this material is effective in the plant as a cofactor for pineapple indoleacetic acid oxidase (Gortner and Kent 1958; Gortner, Kent, and Sutherland 1958). In the absence of this material there is no enzyme activity. In the presence of large amounts of extract, considerable inhibition of enzyme activity is noticed and it is suggested that this effect is due to the ferulic acid ester.

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# THE ALKALOIDS OF *SENECIO JACOBAEA* L.: THE STRUCTURES OF THE ALKALOIDS AND THE NECIC ACIDS

By T. A. GEISSMAN*

[*Manuscript received December* 30, 1958]

*Summary*

Based on a reinterpretation of previously recorded data together with some new evidence, structures VII, IX, XIX, X, and XI have been derived for jacobine, jaconine, jacoline, jaconecic acid, and *iso*jaconecic acid, respectively. The structures of some degradation products of the alkaloids have been clarified.

## I. INTRODUCTION

Earlier work on the alkaloids of *Senecio jacobaea* L. is described in papers that are enumerated by Bradbury and Culvenor (1954) and Adams, Gianturco, and van Duuren (1956). Six alkaloids have been isolated from this plant; in addition to senecionine and seneciphylline (α-longilobine) of known structure, there are present jacobine, jaconine, jacoline, and jacozine (Bradbury and Mosbauer 1956).

The greatest attention has so far been paid to jacobine and jaconine, and the isomeric acids, jaconecic acid and *iso*jaconecic acid, derived from them by alkaline hydrolysis. For these acids, Bradbury and Willis (1956) and Bradbury (1956, 1958, unpublished data†) advanced structures I, II, and III. Adams, Gianturco, and van Duuren (loc. cit.) have proposed an alternative structure IV for jaconecic acid which they obtained from the alkaloid tomentosine of *S. tomentosus*. They considered tomentosine to be a new alkaloid but it is probably identical with otosenine (Santavy 1958) which also yields jaconecic acid on hydrolysis and was isolated from *S. othonnae* and *S. renardii* (Zhdanovich and Menshikov 1944; Danilova and Konovalova 1950).

None of the structures I–IV satisfactorily accounts for all the experimental data that have been reported. The two most striking anomalies are: (i) while jacobine is readily converted to jaconine by the action of dilute hydrochloric acid and reversibly by dilute alkali, and thus appears to be an ethylene oxide derivative, jaconecic acid and *iso*jaconecic acid are stable to prolonged treatment with boiling 15 per cent. hydrochloric acid; (ii) treatment of jaconecic acid with lead tetra-acetate at 100 °C yields carbon dioxide, acetaldehyde, β-methyl-laevulinic acid, and γ-carboxy-β-methyl-γ-valerolactone.

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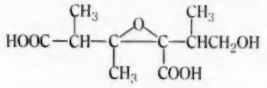
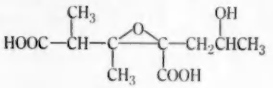
† Unpublished data made available by the Director of the C.S.I.R.O. Chemical Research Laboratories, Melbourne.

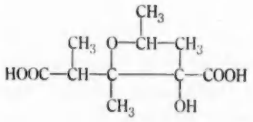
When jacobine or jaconine is hydrolysed with boiling aqueous hydrochloric acid (or jaconine with hydrobromic acid) there is obtained, in addition to retronecine, a chlorodilactone, $C_{10}H_{13}O_4Cl$, the infra-red absorption of which (carbonyl band 1781 $cm^{-1}$ in chloroform; 1770 and 1793 $cm^{-1}$ in "Nujol") led both Adams and Bradbury and Willis to the conclusion that two five-membered



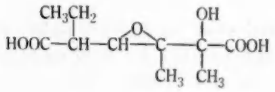
(I) Jaconecic acid (1956) *



(II) Jaconecic acid (1958)



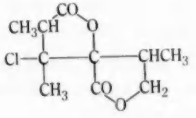
(III) *iso*Jaconecic acid (1958)



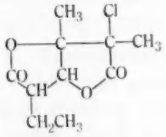
(IV) Jaconecic acid
(Adams, Gianturco, and van Duuren 1956)

* The structure of jacobine would be regarded as that of the cyclic diester formed between the two carboxyl groups of I, II, or IV, and retronecine.

lactone rings were present in the dilactone. This compound, formulated as V by Bradbury and Willis (loc. cit.) and as VI by Adams, Gianturco, and van Duuren (loc. cit.), is converted into jaconecic and *iso*jaconecic acids by alkali, contains no active hydrogen, is unresponsive to catalytic hydrogenation, and contains three carbon-linked methyl groups.



(V)



(VI)

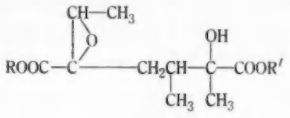
## II. Discussion

A reexamination of the evidence led the present author to the conclusion that no acceptable structure could be devised for jacobine and its hydrolysis products if the requirement were accepted that the chlorodilactone is a γ-lactone. Indeed, the removal of this restriction made it unnecessary to assume a location for the oxide ring of jacobine that would lead to γ-lactone formation, and led

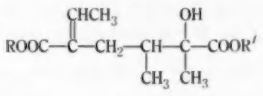
further to the conclusion that while jacobine contains an ethylene oxide ring, jaconecic acid and *iso*jaconecic do not. The evidence for the ethylene oxide–chlorohydrin relationship of jacobine and jaconine is convincing, but no sound evidence exists that jaconecic acid contains the oxirane unit. Infra-red spectrum data were cited by Bradbury and Willis (loc. cit.) to support their contention that the ethylene oxide ring in jacobine (bands at 838, 907, and 1258 $cm^{-1}$ were ascribed to this unit) persists in jaconecic acid (bands at 848, 904, and 1264 $cm^{-1}$), despite the stability of jaconecic acid to vigorous treatment with hydrochloric acid.

### (*a*) *Jacobine and Jaconine*

The structure VII now proposed for jacobine derives from the simple assumption that it is biogenetically closely related to senecionine (VIII), which occurs along with it in the plant:



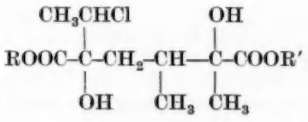
(VII)



(VIII)

R and R′ represent the linkages to retronecine

Jaconine is thus IX, in accordance with the ready interconversion of jaconine and jacobine (Bradbury 1954):



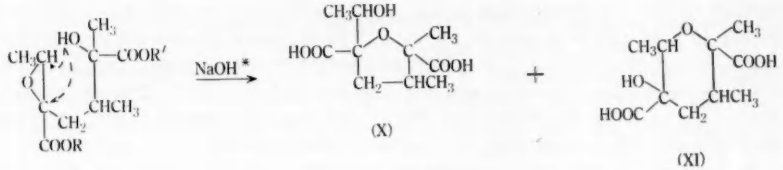
(IX)

The evidence for these structures for jacobine and jaconine is to be found in the way in which they account for the known facts of their conversion into the chlorodilactone, jaconecic acid, and *iso*jaconecic acid and in the chemical evidence for the structures of these derivatives. Further evidence, relevant to jacoline and jacolinecic acid, will be discussed in the sequel.

### (*b*) *Jaconecic and* iso*Jaconecic Acids*

The formation of two isomeric hydroxy acids by the action of alkali upon jacobine* is shown in the following, in which jaconecic acid (X) and *iso*jaconecic acid (XI) are formed by the alkali-induced ring opening of the oxide ring by attack of the 2-hydroxyl group of the alkaloid (see footnote to following diagram):

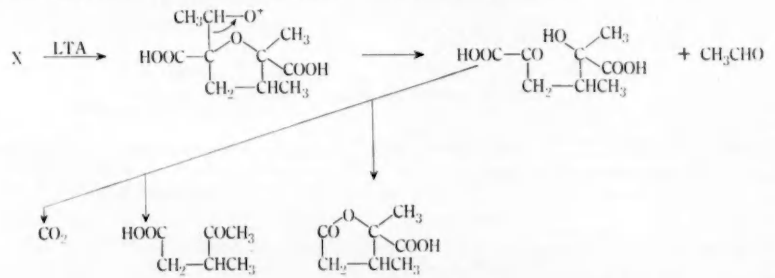
* Or upon jaconine, which is converted into jacobine in a preliminary stage of the alkaline hydrolysis.



(VII) (X) (XI)

* No suggestion is made concerning the sequence of the ester hydrolysis and ring opening steps of the overall reaction.

The experimental data reported by Bradbury (1956, 1958, unpublished data*) are now found to be in complete accord with the structure X for jaconecic acid. Oxidation of jaconecic acid with lead tetra-acetate at 100 °C yields carbon dioxide, acetaldehyde, β-methyl-laevulinic acid, and γ-carboxy-β-methyl-γ-valerolactone. The course of this oxidation is formulated below as proceeding through an initial attack upon the secondary hydroxyl group† and leading to the observed products in the following way:



Nitric acid oxidation of jaconecic acid (Bradbury 1956) yielded αβ-dimethylmalic acid, the origin of which is evident from structure X. In addition to αβ-dimethylmalic acid, two acids, $C_9H_{14}O_6$ and $C_9H_{12}O_7$, were isolated. These acids were not completely characterized, and since their structures are unknown no attempt has been made to accommodate them to the present structure for jaconecic acid.

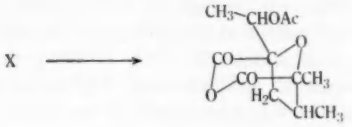
Jaconecic acid is not an α-hydroxy acid;‡ its hydroxyl group can be acetylated, and recent experiments have shown that jaconecic acid yields iodoform when treated with sodium hypoiodite. Vigorous acetylation of

* See footnote, p. 247.

† The writing of the electron-deficient oxygen atom shown in this reaction sequence is to be regarded only as a device, and as the practical equivalent of what may in fact be an intermediate —O—$Pb(OAc)_3$ compound. The subsequent cleavages of the α-keto and α-hydroxy acid intermediates require no comment.

‡ Earlier views that jaconecic acid was an α-hydroxy acid (see Adams, Gianturco, and van Duuren loc. cit.) were based in part upon a positive ferric chloride reaction. Bradbury (1958) later reported that the colour reaction earlier observed must have been due to contamination with *iso*jaconecic acid, which is an α-hydroxy acid, and that pure jaconecic acid gives a negative ferric chloride test.

jaconecic acid gives the acetate of an acid anhydride (Bradbury 1958) now formulated as XII. Mild alkaline hydrolysis of XII yields the acetate of jaconecic acid, which can also be prepared from jaconecic acid by the action of acetyl chloride :

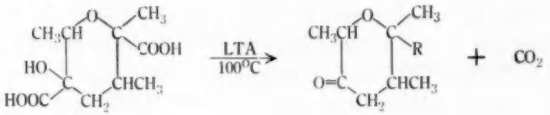


(XII)

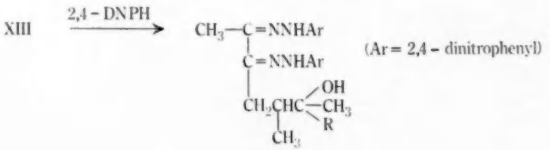
The formation of XII is of interest as an indication of some of the stereochemical features of jaconecic acid, and thus of jacobine.

Reduction of dimethyl jaconecate with lithium aluminium hydride (Bradbury 1958) gave a trihydroxy compound that consumed only 0·2 mole of periodic acid in 48 hr. This result clearly rules out structures that depict jaconecic acid as an α-hydroxy acid, and is in complete accord with structure X.

*iso*Jaconecic acid, on the other hand, is an α-hydroxy acid. It reacts with lead tetra-acetate at 100 °C to give carbon dioxide, some acetaldehyde, and a keto acid (XIII) that contains 9 of the original 10 carbon atoms (Bradbury 1958). The course of this oxidation can now be represented as follows :



(XI) (XIII, R=COOH) (XV, R=$CH_2OH$)



(XIV, R=COOH)
(XVI, R=$CH_2OH$)

The keto acid (XIII) was found to form a semicarbazone in the normal way, but when it was treated with 2,4-dinitrophenylhydrazine (DNPH) the product was a compound which was regarded as the dinitrophenylhydrazone of α-methylacetoacetic acid, despite the fact that attempts to prepare this derivative from that acid gave only the pyrazolone (Bradbury 1958). The reinterpretation of this reaction indicates that the reaction of XIII with 2,4-DNPH involves an

oxidation and leads to the osazone (XIV). The analytical figures for carbon and hydrogen reported for this compound (Bradbury, unpublished data*) are in agreement with those required for XIV.

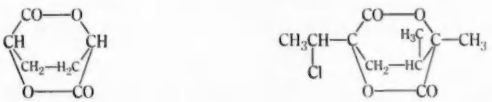
The triol that is formed by the reduction of dimethyl *iso*jaconecate reacts readily with periodic acid, consuming 1·3–1·8 moles of the reagent and giving formaldehyde. The product of this oxidation, the keto alcohol (XV) corresponding to XIII was also found (Bradbury 1958) to react with 2,4-DNPH to give what is now formulated as the osazone (XVI) corresponding to XIV, the analytical figures reported for which are in excellent agreement with those required by structure XVI.

The above review of the published experimental data concerning the behaviour of jaconecic and *iso*jaconecic acids not only gives convincing support to the new formulations for these acids, but allows the assignment of structures to a number of degradation products that it has not heretofore been possible to formulate. It is clear that none of the earlier structures (I–IV) for these acids is adequate to provide so consistent an explanation of these numerous degradation reactions.

### (*c*) *The Chlorodilactone*

The neutral compound that is formed by the hydrolysis of jacobine or jaconine with hydrochloric acid was regarded by both Bradbury and Willis (1956) and by Adams, Gianturco, and van Duuren (1956) as a γ-lactone because of its infra-red absorption at 1781 $cm^{-1}$ in the carbonyl region. It was mentioned above that the only way in which a rational interpretation of the chemistry of jacobine could be achieved was to reject this conclusion and to assume that the chlorodilactone is really the δ-lactone (XVII), formed from jacobine (via jaconine) in the following way:

(VII)



(XVIII) (XVII)

An examination of the literature provided convincing support for this formulation. Marvel *et al.* (1953) measured the infra-red spectrum of the simple model compound XVIII and found it to exhibit a carbonyl band at 1788 $cm^{-1}$ ($CHCl_3$)

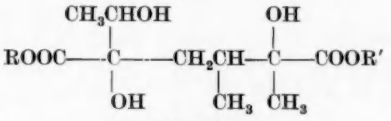
* See footnote p. 247.

and 1784 cm$^{-1}$ (" Nujol "). The wide disparity between the carbonyl absorption of most δ-lactones (about 1745 cm$^{-1}$) and XVII and XVIII is probably due partly to the rigid bicyclic system in the latter compounds and partly to the fact that in XVII and XVIII both carbonyl functions carry α-acyloxy substituents. Other examples of increased carbonyl frequency in the infra-red in bicyclic δ-lactones are known (for another case, see Ashworth, Whitham, and Whiting 1957).

With the discovery that the carbonyl peak at 1781 cm$^{-1}$ for the chlorodilactone is indeed consistent with the structure XVII, there remains no compelling reason to retain in consideration structures for jacobine that can give rise to γ-lactone formation.

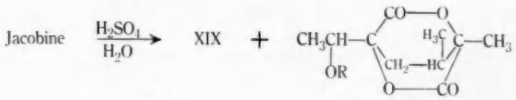
### (*d*) *Jacoline*

Jacoline has the composition $C_{18}H_{27}O_7N$, corresponding to the addition of water to jacobine. An early observation of Bradbury's (unpublished data*), that jacoline consumed 1 mole of periodic acid, with the formation of acetaldehyde, is entirely in agreement with the simple assumption that jacoline is the glycol (XIX) corresponding to the ethylene oxide, jacobine:



(XIX)

Experiment has confirmed this. When jacobine was hydrolysed with aqueous sulphuric acid, jacoline was formed in good yield together with a neutral compound the acetyl derivative of which is evidently the acetoxydilactone (XXI) corresponding to the chlorodilactone (XVII).



(XX, R = H)
(XXI, R = Ac)

## III. Conclusion

The above consideration of earlier experimental data, coupled with the new evidence, fully substantiates the proposed structures for jacobine, jaconine, jacoline, jaconecic acid, and *iso*jaconecic acid. Further work is in prospect to establish the stereochemistry of these compounds and to determine the manner in which these dibasic acids are attached to the hydroxyl groups of retronecine in the alkaloids.

* See footnote, p. 247.

## IV. Experimental

(*a*) *Iodoform Test.*—To a sample of jaconecic acid dissolved in 6N aqueous sodium hydroxide was added a solution of iodine in aqueous potassium iodide until the iodine colour persisted. After a few minutes iodoform was deposited as a yellow, crystalline precipitate. It was collected and recrystallized from aqueous methanol. It was identified by its m.p. and mixed m.p. with authentic material.

(*b*) *Jacoline.*—A solution of 2·0 g of pure jacobine in 25 ml of 6N sulphuric acid was refluxed for 2 hr and then kept at 70 °C overnight. The solution was cooled and extracted with ether and with chloroform. Evaporation of the combined extracts yielded 0·32 g of a pale yellow oil. This was acetylated by refluxing it for 30 min with acetyl chloride. Removal of the acetyl chloride left an oil that soon crystallized. Recrystallized from ethyl acetate–light petroleum, the product formed tiny white prisms, m.p. 157–158 °C. Bradbury and Willis (loc. cit.) report the m.p. of acetyljacolinecic lactone as 159–160 °C (Found: C, 56·1; H, 6·3%. Calc. for $C_{12}H_{16}O_6$: C, 56·3; H, 6·3%).

The aqueous acidic solution, after removal of the lactone, was made alkaline and the crystalline precipitate taken up by extraction with chloroform. The residue that remained after removal of the chloroform was recrystallized from ethanol. It had m.p. 216–218 °C, and the mixture with authentic jacoline (m.p. 217–219 °C) melted at 217–219 °C. The mixed m.p. with jacobine (m.p. 220–221 °C) was 195–205 °C.

## V. Acknowledgments

The author wishes to express his gratitude to members of the C.S.I.R.O. Chemical Research Laboratories, particularly Dr. J. R. Price, for their kindness in offering him the hospitality of their laboratories during his tenure of a Fulbright Research Scholarship, for affording him the facilities and material for carrying on this study, and for helpful discussions during its course.

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# 1-METHYLENEPYRROLIZIDINE, THE MAJOR ALKALOID OF *CROTALARIA ANAGYROIDES* H.B. & K.

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*Summary*

The principal alkaloid of *Crotalaria anagyroides* H.B. & K. has been identified as 1-methylenepyrrolizidine.

## I. Introduction

*Crotalaria anagyroides* H.B. & K. is a perennial shrub used as cover crop on coffee plantations in New Guinea. Total alkaloid assay and paper chromatography disclosed the presence of at least three bases of $R_F$ 0·28, 0·43, and 0·56, with *N*-oxides forming only a minor part of the alkaloid present. The levels of tertiary base and *N*-oxide found in whole plant and seed are shown in Table 1. The principal component, $R_F$ 0·28, is readily separated from the others by virtue of its volatility in steam. A hygroscopic liquid, it forms a crystalline picrate and picrolonate and has the empirical formula $C_8H_{13}N$.

Table 1

ALKALOID CONTENT OF CROTALARIA ANAGYROIDES SAMPLES

| Plant Part | Tertiary Base (%) | *N*-Oxide (%) |
|---|---|---|
| Leaf and stem | | |
| First sample .. .. | 0·13 | 0·06 |
| Second sample .. .. | 0·35 | n.d. |
| Seed .. .. .. .. | 1·5 | 0·03 |

In the presence of Raney nickel this base absorbed 1 mole of hydrogen to give a dihydro derivative whose properties, excepting optical rotation, were very similar to those of heliotridane (II). Infra-red spectra of the two bases and of their picrates showed only slight differences (Figs. 1, 2). The dihydro derivative was clearly a mixture of heliotridane and pseudoheliotridane (III) but complete proof of this point was not achieved until near the end of the investigation when pure pseudoheliotridane was isolated from the mixture (see below). Since all previously known alkaloids with an unsaturated pyrrolizidine ring have the double bond in the 1,2-position, it seemed probable that the base was 1-methyl-1,2-dehydropyrrolizidine (IV). The properties of IV, previously

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prepared by Adams and Mahan (1943) by removing the hydroxyl group from deoxyretronecine (V) and known as *iso*heliotridene, were sufficiently close to those of the alkaloid to make direct comparison desirable. IV was obtained from supinidine (VI) by converting this compound into 1-chloromethyl-1,2-dehydropyrrolizidine (VII) and reducing the latter with zinc dust. It was found to resemble the alkaloid fairly closely and the infra-red spectra of the picrates (Fig. 1) were almost identical. Small differences in optical rotation and picrate melting point could be accounted for on the hypothesis that the alkaloid isolated from *C. anagyroides* was a mixture of the unsaturated compound with

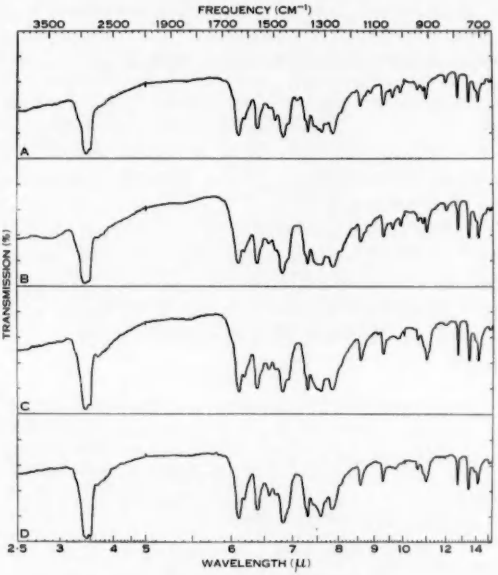
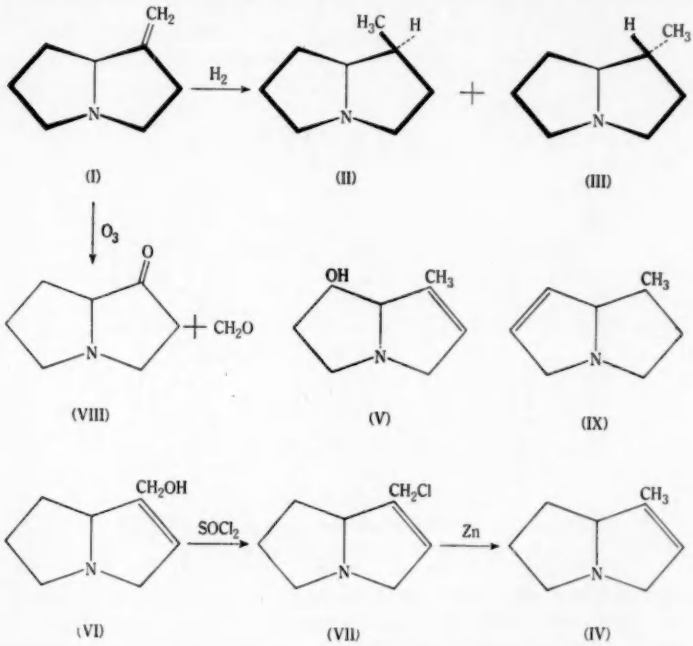



Fig. 1.—Infra-red spectra of picrates.
*A*, Heliotridane; *B*, dihydro derivative of 1-methylenepyrrolizidine; *C*, 1-methylenepyrrolizidine; *D*, *iso*heliotridene.

about 25 per cent. of pseudoheliotridane. Repeated efforts were therefore made to resolve it into two such components. Various chromatographic procedures, including gas partition chromatography, failed to demonstrate inhomogeneity although a synthetic mixture of IV and heliotridane was partially resolved on a partition column buffered at pH 7·5. The base was finally recognized to be a pure compound and three further observations showed that IV could not be a major component: (i) the picrolonates of the alkaloid and IV differed considerably in melting points which were depressed on admixture; (ii) the infra-red spectra of the liquid bases showed that IV was not present in the alkaloid (Fig. 2; a band at 1385 $cm^{-1}$ in the spectrum of IV is missing in that of the alkaloid);

and (iii) IV was found to be reduced catalytically to pure heliotridane (as reported by Adams and Mahan 1943). The last observation means that a mixture of IV with a minor proportion of pseudoheliotridane would not yield on hydrogenation a mixture rich in the latter as is obtained from the alkaloid. With this particular group of compounds it became clear that picrates are unreliable characterizing derivatives; their mixed melting points and infra-red spectra may not give a definite indication of small structural differences. In this regard, the picrolonates are superior; in all instances encountered, mixed melting points between different




picrolonates were substantially depressed, and heliotridane and pseudoheliotridane picrolonates, for example, show significant differences in infra-red absorption (Fig. 3).

The other likely structure for the alkaloid, 1-methylenepyrrolizidine (I), was readily established. Microanalysis showed the absence of a *C*-methyl group, and on ozonolysis, formaldehyde was formed together with a basic product, $C_7H_{11}ON$, which is evidently 1-ketopyrrolizidine (VIII). Proof that the methylene group is attached at $C_1$, rests on the definite identification of pseudoheliotridane as one component of the mixture resulting from hydrogenation. Repeated chromatography of the hydrogenation product on alumina led eventually to pseudoheliotridane of constant specific rotation ($-2{\cdot}5°$ in ethanol) and picrolonate melting point (172–173·5 °C). The picrolonate neither depressed

nor elevated the melting point of the authentic *d*-base derivative, kindly supplied by Professor N. J. Leonard, and its infra-red spectrum was identical with that of the authentic *d*-compound (Fig. 3). Heliotridane was not obtained in a pure state from the mixture. Galinovsky, Vogl, and Nesvadba (1954) have recorded

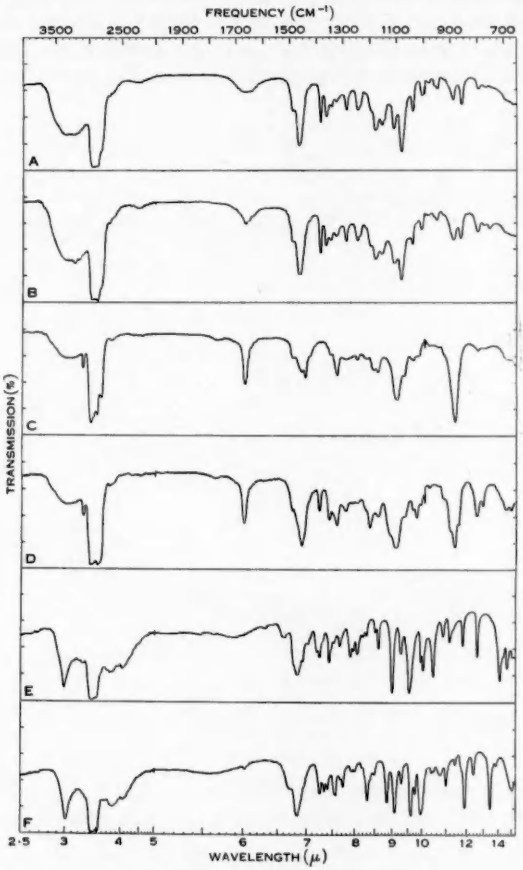



Fig. 2.—Infra-red spectra of free bases.

*A*, Heliotridane; *B*, dihydro derivative of 1-methylenepyrrolizidine; *C*, 1-methylenepyrrolizidine; *D*, *iso*heliotridene; *E*, heliotridine; *F*, retronecine.

a value, $+17{\cdot}1°$, for the specific rotation in ethanol solution of *d*-pseudoheliotridane derived from laburnine, which differs widely from the value here obtained for the *l*-compound. As the end result of a series, decreasing in magnitude as purification proceeded, the present value seems reliable and we can suggest only

that the sample of laburnine used by Galinovsky, Vogl, and Nesvadba (1954) was not pure. Since the absolute configuration of heliotridane has been established by Warren and von Klemperer (1958) as the form depicted in II, it follows that I represents the absolute configuration of (—)-1-methylenepyrrolizidine whose systematic name is (8*S*)-1-methylenepyrrolizidine.

1-Methylenepyrrolizidine has a strong absorption band at 880 $cm^{-1}$ (Fig. 2), which is close to the range (885–895 $cm^{-1}$) reported by Bellamy (1958) for the CH out-of-plane deformation frequency of the grouping $CRR=CH_2$. However, this band has limited diagnostic value in this series since *iso*heliotridene (IV)

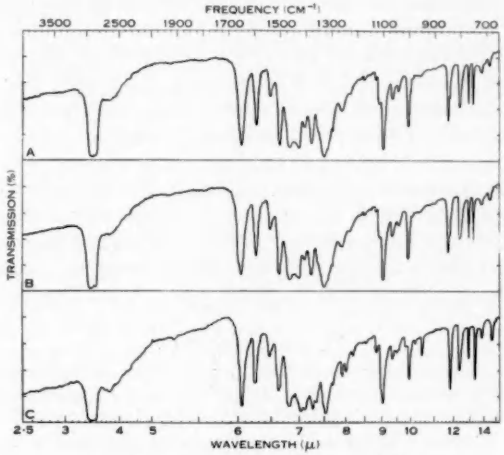



Fig. 3.—Infra-red spectra of picrolonates.
*A*, *l*-Pseudoheliotridane (present work); *B*, *d*-pseudoheliotridane (Leonard); *C*, heliotridane.

also shows a strong band at 880 $cm^{-1}$ (Fig. 2). Two other pyrrolizidines with a double bond situated as in IV, retronecine and heliotridine, show no strong absorption at this point (Fig. 3). Heliotridine and retronecine are unusual in not showing specific absorption in the region of C=C stretching frequencies (1620–1680 $cm^{-1}$).

During preparation of heliotridene (IX) for comparative infra-red studies, an explanation was found for the great discrepancy between published values for the specific rotation of this compound; in one instance, the need to consider both the *dextro*- and the *laevo*-readings of a polarimeter setting had been overlooked. Konovalova and Orekhov (1936, 1937) reported $[\alpha]_D$ −160° and −150° (pure liquid) for two samples prepared by sulphuric acid dehydration of hydroxyheliotridane and retronecanol, respectively. For a sample prepared similarly from retronecanol, Adams and Rogers (1941) reported +39°, changing on keeping to +31°. The latter authors used a 2 dm tube, giving polarimeter readings of +72·5° and +57·5° for their two determinations. These readings could also

have been taken as $-287 \cdot 5°$ and $-302 \cdot 5°$, which correspond to specific rotations of $-154°$ and $-162°$ in reasonable agreement with the data of the Russian authors. The sample prepared here (by the same procedure) was too small to check the rotation of the pure liquid but it had $[\alpha]_D$ $-171°$ in ethanol solution. There is little doubt, therefore, that the negative rotation is the true one. A similar ambiguity was found to exist for chlororetronecane, obtained during the present work as an intermediate in the preparation of heliotridane. For this compound, Adams and Rogers (1941) recorded $[\alpha]_D$ $+53 \cdot 79°$ (pure liquid); their data could also yield the value $-31 \cdot 5°$. Our sample, which crystallized, had $[\alpha]_D$ $-30°$ as supercooled liquid, and $-4 \cdot 7°$ in ethanol solution. These two examples emphasize the need for care in measuring the specific rotation of pure liquids of substantial rotatory power; for these compounds more than one of the four possible values of $[\alpha]$ for a given setting of the polarimeter may have to be considered if a tube of 1 or 2 dm is used. The ambiguity is removed by determining the rotation with two or more tubes of different lengths, but more conveniently by using a very short tube, e.g. 0·25 dm.

The present work brings to five, the number of known occurrences of non-ester pyrrolizidines in the genus *Crotalaria*. Those previously found are retronecine *N*-oxide in *Crotalaria retusa* (Culvenor and Smith 1957), 1-methoxymethyl-1,2-dehydropyrrolizidine and a second base from *C. trifoliastrum* and *C. aridicola*, and 7-hydroxy-1-methyl-1,2-dehydropyrrolizidine from *C. goreensis* (Culvenor 1958). Such bases may be more common than has hitherto been supposed.

## II. Experimental

Microanalyses were made by the C.S.I.R.O. and Melbourne University Microanalytical Laboratory. Melting points are corrected. The solvent used for paper chromatography was the upper phase resulting from shaking butanol with an equal volume of 5% acetic acid.

(*a*) *Total Alkaloid Assays.*—The method used was essentially that of Culvenor and Smith (1955). However, when the seed assay was performed, the volatility of the alkaloid was known and ether was used instead of chloroform as extracting solvent. Ether, also, was not entirely suitable since extraction was slow and the product contained a considerable amount of water. Light petroleum (b.p. $<40$ °C) was found to be the best extracting solvent.

(*b*) *Isolation of the Principal Alkaloid.*—In addition to the assay extractions which followed the usual methanol–dilute acid–chloroform (or ether) route, a steam-distillation procedure was tried. Milled seed (104 g) was steam-distilled directly with 1 l. of 0·2% $Na_2CO_3$ solution. Two 700 ml distillate fractions were collected and separately treated with excess $NH_3$ and extracted with ether; the first gave 1·5 g and the second 0·05 g base. The total yield, 1·35%, is slightly lower than in the normal assay. The difference presumably represents non-volatile bases. Crude base obtained by methanol extraction and showing $R_F$ values, 0·13, 0·31 (major component), 0·38, 0·44, was also steam-distilled from $Na_2CO_3$ solution under reduced pressure. Base showing only 1 spot, $R_F$ 0·30, was recovered in good yield from the distillate. The residue from the steam-distillation was extracted with chloroform to give a very small amount of base, $R_F$ 0·05, 0·20, 0·36, indicating that the minor components may be partially destroyed during steam-distillation.

Available crude base was combined and fractionally distilled. A small fraction (0·25 g) came over with the thermometer indicating 86 °C (at 760 mm), but nearly all the remainder (2·9 g) distilled at 120 °C/175 mm. This main portion was collected in 3 fractions; specific rotations were $-43 \cdot 6°$, $-45 \cdot 0°$, and $-45 \cdot 0°$ (in ethanol). The third fraction had $[\alpha]_D^{20}$ $-55 \cdot 7°$ as pure liquid. The middle portion run on a gas chromatographic column packed with " Surf " (detergent powder consisting essentially of sodium dodecylbenzenesulphonate) at 180 °C showed a single

peak eluted in 7·4 min. Redistillation gave the base as a mobile oil, b.p. 120 °C/170 mm, $[\alpha]_D^{20}$ —43·1° (*c*, 1·07 in ethanol) (Found : C, 76·3 ; H, 10·6 ; N, 11·3 ; (C)Me, 0·7%. Calc. for $C_8H_{13}N+2\%\ H_2O$ : C, 76·5 ; H, 10·5 ; N, 11·2 ; 1×(C)Me, 12·2%). The base and its dihydro derivative are hygroscopic and difficult to obtain free from water ; OH absorption is evident in their infra-red spectra (Fig. 2). The ultraviolet spectrum showed a single small peak at 2130 Å (*ε*, 174).

The *picrate* formed needles from ethanol (m.p. 217·5–218 °C, $[\alpha]_D^{20}$ —7·3° (*c*, 1·1 in ethanol) (Found : C, 47·5 ; H, 4·8 ; N, 15·5%. Calc. for $C_8H_{13}N.C_6H_3O_7N_3$ : C, 47·7 ; H, 4·6 ; N, 15·9%). The same picrate was obtained from the low boiling material, probably aqueous alkaloid, obtained in the fractionation described above.

The *picrolonate* formed needles from ethanol, m.p. 171·5–172·5 °C (Found : C, 55·5 ; H, 5·2 ; N, 17·5%. Calc. for $C_8H_{13}N.C_{10}H_8O_5N_4$ : C, 55·8 ; H, 5·4 ; N, 18·1%).

The $pK_a$ of the alkaloid was determined as 10·2 ; those of *iso*heliotridene and heliotridane were found to be 10·2 and 11·0 respectively (Adams, Carmack, and Mahan (1942) give 10·2, 11·5 respectively). The titration curve was that of a homogeneous base. Chromatography of the alkaloid on glass powder columns buffered at various pH's from 10·7 to 7·5, resulted in all the material being eluted in a single sharp peak. When a mixture of *iso*heliotridene and heliotridane was chromatographed similarly at pH 7·5, partial resolution occurred and some pure heliotridane was obtained from a second peak.

(*c*) *Hydrogenation of the Alkaloid.*—In dilute $H_2SO_4$, with PtO catalyst, the base absorbed 1 mole of hydrogen. Recovery with light petroleum (b.p. <40 °C) gave an oil distilling at 120 °C/180 mm, $[\alpha]_D^{20}$ —18·6° (*c*, 1·46 in ethanol), $R_F$ 0·30. The picrate formed needles from ethanol, $[\alpha]_D^{20}$ —4·3° (*c*, 1·1 in acetone), m.p. 248–249 °C (decomp.), undepressed on admixture with heliotridane picrate (Found : C, 47·7 ; H, 5·2 ; N, 15·5%. Calc. for $C_8H_{15}N.C_6H_3O_7N_3$ : C, 47·5 ; H, 5·1 ; N, 15·8%). The picrolonate had a melting point varying from 146 to 152 °C, but it depressed the m.p. of heliotridane picrolonate by 12 °C. For comparison, the properties of heliotridane and pseudoheliotridane reported in the literature and determined in the present work are recorded in Table 2. The hydrogenation product is apparently composed of about 26% heliotridane, 74% pseudoheliotridane. Despite this, its infra-red spectrum could barely be distinguished from that of heliotridane (Fig. 2). A second experiment, using Raney nickel as catalyst, gave an almost identical product, $[\alpha]_D^{20}$ —19·7° (*c*, 2·5 in ethanol).

An attempt to resolve the hydrogenation product into pure components on a partition column buffered at pH 8·0 failed. A partial separation was achieved on neutral alumina with light petroleum (b.p. 40 °C) and ether as eluants, progress being followed by determining the specific rotation in ethanol of each eluate fraction. Light petroleum eluted about 25% of the pseudoheliotridane as a mixture containing up to about 10% heliotridane ; the remainder, eluted by light petroleum–ether mixtures, contained larger amounts of heliotridane. No fraction contained more than 50% heliotridane. After rechromatographing several times, all fractions with laevorotations (in ethanol) of magnitude <8° were combined and converted into picrolonate. Crystallization from ethanol gave a constant m.p. 172·5–173·5 °C, $[\alpha]_D^{20}$ +3·2±1·0° (*c*, 1·08 in ethanol). *d*-Pseudoheliotridane picrolonate, kindly supplied by Professor N. J. Leonard, had m.p. 167–168 °C; a mixture melted at 167·5–169 °C. The infra-red spectra of the two picrolonates were identical and differed from the spectrum of heliotridane picrolonate mainly in having an absorption peak at 1115 $cm^{-1}$ and in not having peaks at 955 and 1020 $cm^{-1}$ (Fig. 3).

*Pseudoheliotridane*, recovered from the pure picrolonate had $[\alpha]_D^{20}$ —2·5±0·5° (*c*, 1·81 in ethanol). Reported constants for pseudoheliotridane are given in Table 2. A sample of *d*-pseudoheliotridane picrate also supplied by Professor Leonard, was found to have $[\alpha]_D^{18}$ +0·5° (*c*, 1·44 in acetone).

(*d*) *1-Chloromethyl-1,2-dehydropyrrolizidine* (*VII*).—Supinidine was warmed with excess thionyl chloride for 30 min before removing the thionyl chloride under reduced pressure. The crude hydrochloride of 1-chloromethyl-1,2-dehydropyrrolizidine thus obtained did not crystallize and was used as such ; when converted into free base by neutralizing in solution, extracting with

chloroform, and evaporating, the resulting oil rapidly changes into a solid quaternary polymer soluble in water but not in organic solvents. The hydrochloride yielded a *picrate*, needles from ethanol, m.p. 176·5–177·5 °C (Found: C, 43·5; H, 3·9; Cl, 9·6%. Calc. for $C_8H_{12}NCl.C_6H_3O_7N_3$: C, 43·5; H, 3·9; Cl, 9·2%).

(*e*) *1-Methyl-1,2-dehydropyrrolizidine* (*IV*).—The crude hydrochloride of VII was taken up in 2N $H_2SO_4$ and shaken with zinc dust for 3 hr. The base recovered distilled at 100 °C/110 mm and had $[\alpha]_D^{20}$ —66·5° (*c*, 2·38 in ethanol). It was purified by conversion to picrate, which formed plates from ethanol, m.p. 205–206 °C, $[\alpha]_D^{20}$ —12·7° (*c*, 1·0 in acetone) (Found: C, 47·8; H, 4·5; N, 15·5%. Calc. for $C_8H_{13}N.C_6H_3O_7N_3$: C, 47·7; H, 4·5; N, 15·9%). Base recovered from this picrate and distilled had $[\alpha]_D^{20}$ —57·6° (*c*, 0·95 in ethanol). The picrate has a

TABLE 2

PHYSICAL PROPERTIES OF HELIOTRIDANE AND PSEUDOHELIOTRIDANE

| Property | Heliotridane | | Pseudoheliotridane | |
|---|---|---|---|---|
| | Literature | Present Work | Literature | Present Work |
| Specific rotation, pure liquid | —68° (Menshikov 1933)<br>—92° (Adams & Rogers 1941)<br>—99·5° (Menshikov 1935) | | +6·9°* (Leonard & Felley 1950)<br>—8·25° (Menshikov & Borodina 1945) | |
| Specific rotation, ethanol solution | —53° (Konovalova & Orekhov 1936) | —64·4° | +17·1* (Galinosvky, Vogl, & Nesvadba 1954) | —2·5° |
| Picrate, specific rotation in acetone | | —20·4° | | +0·5°† |
| Picrate, m.p. (°C) | 236 (Menshikov 1933, 1935; Adams & Rogers 1941)<br>243–244 (Leonard & Felley 1950)<br>237–238 (Konovalova & Orekhov 1936) | 248·5–249·5 | 232–233 (Menshikov & Borodina 1945)<br>234–236 (Leonard & Felley 1950)<br>234–235 (Galinovsky, Vogl, & Nesvadba 1954) | |
| Picrolonate, m.p. (°C) | 152–153 (Konovalova & Orekhov 1936, 1937) | 154–154·5 | 165 (Galinovsky, Vogl, & Nesvadba 1954) | 172–173·5 |

* Measurement on *d*-pseudoheliotridane.

† Sample from Professor Leonard.

different crystalline form from that of 1-methylenepyrrolizidine and is much less soluble in alcohol; nevertheless, a mixture of the two melts at an intermediate temperature. Synthetic mixtures of the picrates of IV (75%) and heliotridane, and IV and the dihydro derivative of the alkaloid, had m.p.'s 219–220 °C and 217–218 °C, respectively, and neither depressed the m.p. of the alkaloid picrate.

*1-Methyl-1,2-dehydropyrrolizidine picrolonate* formed plates from ethanol, m.p. 185–186 °C (Found: C, 55·5; H, 5·6; N, 17·8%. Calc. for $C_8H_{13}N.C_{10}H_8O_5N_4$: C, 55·8; H, 5·4; N, 18·1%).

(*f*) *7-Chloro-1-methylpyrrolizidine* (*chlororetronecane*).—Retronecanol (2 g) was added slowly to ice-cooled thionyl chloride (3·2 ml), kept for 1 hr, heated at 100 °C for 45 min, cooled, and poured into ice-water. The solution was basified and extracted with ether, ether removed from the extract, and the residual oil (1·4 g) distilled to give 7-chloro-1-methylpyrrolizidine, b.p. 104 °C/25 mm. The main fraction (0·7 g) crystallized when touched with a dropper tube; this

material could not be recrystallized but in bulk melted at about 33 °C. Measurements of optical rotation were made by slightly warming the base and polarimeter tube, filling and sealing the tube, and then taking the measurement on the supercooled liquid. The density value 1·055, of Adams and Rogers (1941) was used. Two different preparations gave results as follows: (i) α, —20°; *l*, 0·5 dm; $[\alpha]_D^{20}$ —38°; (ii) α, —41°; *l*, 1 dm; $[\alpha]_D^{20}$ —39°. For an ethanol solution (*c*, 1·07), $[\alpha]_D^{20}$ —4·7° was observed. To ensure purity, the compound was converted into picrate, needles from ethanol, m.p. 212–213 °C (Found: C, 43·3; H, 4·4; N, 14·1; Cl, 9·0%. Calc. for $C_8H_{14}NCl.C_6H_3O_7N_3$: C, 43·2; H, 4·4; N, 14·4; Cl, 9·1%). The base was recovered from the picrate by passage through a column of "Deacidite FF", and redistilled. It then gave α, —8·0±0·1°; *l*, 0·25 dm; $[\alpha]_D$ —30·3°.

(*g*) *Heliotridane.*—7-Chloro-1-methylpyrrolizidine was reduced with hydrogen and Raney nickel and the product converted into picrate and recrystallized from ethanol to give needles, m.p. 248·5–249·5 °C (decomp.), $[\alpha]_D^{20}$ —20·4° (*c*, 0·89 in acetone) (Found: C, 47·7; H, 5·1; N, 15·2%. Calc. for $C_8H_{15}N.C_6H_3O_7N_3$: C, 47·5; H, 5·1; N, 15·8%). Recovery of base from this picrate ("Deacidite FF") and distillation (bath temp. 105–110 °C/130 mm) gave heliotridane, $[\alpha]_D^{20}$ —64·4° (*c*, 1·76 in ethanol). The picrolonate crystallized from ethanol in needles, m.p. 154–154·5 °C (Found: C, 55·4; H, 5·8; N, 17·6%. Calc. for $C_8H_{15}N.C_{10}H_8O_5N_4$: C, 55·5; H, 5·9; N, 18·0%). For constants reported in the literature see Table 2.

(*h*) *1-Methyl-6,7-dehydropyrrolizidine (heliotridene).*—Retronecanol was heated with conc. $H_2SO_4$ for 1 hr at 150 °C, and the product recovered in the usual way and distilled (bath temp. 160–170 °C). The resulting base was converted into picrate, needles from ethanol, m.p. 236·5–237 °C (decomp.), $[\alpha]_D^{20}$ —25·2° (*c*, 1·43 in acetone) (Found: C, 47·7; H, 4·6; N, 15·9%. Calc. for $C_8H_{13}N.C_6H_3O_7N_3$: C, 47·7; H, 4·5; N, 15·9%). Recovered from this picrate ("Deacidite FF") and distilled, the base had $[\alpha]_D^{20}$ —171° (*c*, 0·59 in ethanol). The *picrolonate* crystallized in plates from ethanol, m.p. 179–179·5 °C (Found: C, 55·8; H, 5·6; N, 18·1%. Calc. for $C_8H_{13}N.C_{10}H_8O_5N_4$: C, 55·8; H, 5·4; N, 18·1%). Konovalova and Orekhov (1937) record $[\alpha]_D$ —149·8° (pure liquid), picrate, m.p. 224–225 °C, picrolonate, m.p. 175–176 °C, whereas Adams and Rogers (1941) give $[\alpha]_D$ +39° (pure liquid) picrate, m.p. 224–225 °C (see Section I).

(*i*) *Ozonolysis of the Alkaloid.*—Excess ozone was bubbled through a solution of the base (0·2 g) in dilute HCl for 3 hr. The issuing gas was bubbled through a solution of dimedone in 50% aqueous ethanol but no precipitate formed. The main reaction mixture was divided into 2 portions. One was treated with 2,4-dinitrophenylhydrazine and gave a precipitate, m.p. 165·5–166 °C (after crystallization from ethanol), mixed m.p. 166–167 °C with authentic formaldehyde 2,4-dinitrophenylhydrazone. The other portion, with aqueous picric acid gave *1-ketopyrrolizidine picrate*, yellow needles, m.p. 172·5–173·5 °C, from ethanol (Found: C, 44·3; H, 4·0; N, 15·5%. Calc. for $C_7H_{11}ON.C_6H_3O_7N_3$: C, 44·1; H, 4·0; N, 15·8%).

## III. Acknowledgments

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# CHEMISTRY OF NON-ENZYMIC BROWNING*

## III. EFFECT OF BISULPHITE, PHOSPHATE, AND MALATE ON THE REACTION OF GLYCINE AND GLUCOSE

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*Summary*

1-Deoxy-1-glycino-D-fructose was obtained in good yield from the reaction of glycine and glucose in the presence of sodium bisulphite and 10 per cent. of water. Small quantities of by-products with similar properties were formed. The results were not affected qualitatively by the reaction temperature or the presence of salts of phosphoric or malic acids.

For quantitative studies deoxyglycinofructose was separated from neutral reducing compounds on a cation-exchange resin and determined with alkaline ferricyanide.

The yield of deoxyglycinofructose was dependent on the proportion of glucose and bisulphite to glycine and decreased as the water content of the mixture was increased above 10 per cent.

The rate of reaction of glycine and glucose in the presence of bisulphite was studied over the pH range 3·5–5·6 and the temperature range 25 to 100 °C. Pseudo first-order rate constants obtained for pH 3·5 and 4·7 fitted the Arrhenius equation.

The addition of phosphate at pH 3·6 and malate at pH 3·5 increased the rate of reaction of glycine and glucose; the increase was proportional to the square root of the concentration of dihydrogen phosphate or hydrogen malate ion.

The implications of these results in the non-enzymic browning of dried fruit are discussed.

### I. INTRODUCTION

It is usually considered that non-enzymic browning in foodstuffs is initiated by the reaction of amino compounds with reducing sugars and it has been shown (Anet and Reynolds 1957) that browned freeze-dried fruit contains 1-*N*-(amino acid)-1-deoxyfructoses, the products of the reaction between amino acids and glucose. Browning in dried fruit is inhibited by the addition of sulphur dioxide or bisulphites but the nature of the chemical reactions involved is not known. It is now shown that the presence of sodium bisulphite does not affect the nature of the reaction between glycine and glucose. In fact the reaction of amino acids and sugars in the presence of sodium bisulphite has proved to be a convenient method of synthesizing amino acid-deoxyfructoses and applications of the method have already been described (Anet and Reynolds 1957; Anet 1957). This paper also presents the results of some kinetic studies of the reaction between glycine and glucose, in mixtures containing 75–80 per cent. solids, in the presence of bisulphite, phosphate, or malate.

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## II. Effect of Bisulphite

It was found that glucose readily gave a syrup when heated at 100 °C with 10 per cent. of water. Glycine and sodium metabisulphite dissolved rapidly in the hot syrup and, in preparative experiments, heating at 100 °C was continued for about an hour. The reaction products were separated by displacement chromatography using a set of columns of a cation-exchange resin. Crystalline 1-deoxy-1-glycino-D-fructose, identical with the compound isolated in the absence of bisulphite (Anet 1957), was obtained in 33 per cent. yield. Paper chromatography showed that some fractions contained small proportions of by-products (U1–U6). U1 was the main by-product; there were only trace amounts of U2–U4. The compounds U1, U5, and U6 gave the same reactions as deoxyglycinofructose and corresponded with the by-products (F*a*, F*b*, F*c*) formed generally from the reaction of amino acids and sugars (Anet and Reynolds 1957, p. 190) including those from aspartic acid and asparagine which were detected (Anet and Reynolds 1957) in browned freeze-dried fruit. This, together with the fact that they were held on an unbuffered cation-exchange resin, appears to prove that none of these compounds were sulphonic acid derivatives. The compound called U1 has now been obtained pure and crystalline and shown to be *NN*-di-(D-*arabino*-3,4,5,6-tetrahydroxy-2-oxohexyl)glycine (trivial name, difructose-glycine) (Anet 1958).

Deoxyglycinofructose and the same by-products, except U3 and U4, were formed when the reaction was carried out at 25, 50, or 75 °C, or at 100 °C in the presence of potassium hydrogen malate or sodium dihydrogen phosphate. Failure to detect U3 and U4 is not significant in view of the smaller scale of these experiments.

For quantitative studies the basic components of the reaction mixtures were collected on a column of a cation-exchange resin which was then washed until free from sulphurous acid and glucose. The bases were eluted with acid or alkali and their reducing value was determined with alkaline ferricyanide and arsenomolybdate under conditions which were essentially those described by Ting (1956) for the determination of reducing sugars. The results were expressed as percentage yield of deoxyglycinofructose. The values would include the by-products but, in view of the small proportions seen in displacement chromatograms, it was considered that this error could be disregarded in the work now described.

The results of a preliminary study of the effect of varying the proportions of glucose and bisulphite, in mixtures heated for 1 hr at 100 °C, are shown in Table 1. For mixtures of similar pH, there was an increase in the yield of deoxyglycinofructose with both increasing proportions of glucose and decreasing proportions of bisulphite. The ratio of free amino acid to glucose and sulphur dioxide (or bisulphite) likely to be present in dried apricots is 11 : 80 : 5·6, these figures being derived from the values for amino acid and glucose found by Anet and Reynolds (1957) and the legally permissible sulphur dioxide content. This is close to the 1 : 8 : 1 (glycine : glucose : bisulphite) mixture which gave one of the highest yields of deoxyglycinofructose.

This 1 : 8 : 1 mixture was heated for 1 hr at 100 °C with different quantities of water. The yield of deoxyglycinofructose decreased from 53 to 26 per cent. as the water content of the mixture was increased from 10 to 45 per cent. Hannan and Lea (1952) found that the rate of loss of amino nitrogen in a mixture of glucose and α-*N*-acetyl-L-lysine was greatest with 8 per cent. water content.

The freeze-dried fruit previously studied contained 21 per cent. of water and some kinetic studies were started with the 1 : 8 : 1 mixture and the lowest practicable water content (24 per cent.). The mixture remained syrupy indefinitely at 49·5 °C but glucose slowly crystallized at 37·5 °C and part of the glucose was replaced by sorbitol in studying the effect of temperature. The

TABLE 1

EFFECT OF PROPORTIONS OF REACTANTS ON YIELD OF 1-DEOXY-1-GLYCINOFRUCTOSE*†

Reaction mixture: Glycine (1 mole) with glucose and sodium metabisulphite as presented; water content 10%; heated 1 hr at 100 °C

| Sodium Bisulphite‡ (moles) \ Glucose (moles) | 1 | 2 | 4 | 8 | 16 |
|---|---|---|---|---|---|
| 0·25 | — | — | — | — | 58 (5·8) |
| 0·5 | — | — | — | — | 50 (5·7) |
| 1 | — | — | 52 (5·5) | 53 (5·6) | 48 (5·7) |
| 2 | 10 (2·5) | 28 (5·3) | 39 (5·4) | 40 (5·4) | 40 (5·5) |
| 4 | — | 18 (5·3) | 28 (5·3) | 31 (5·2) | 34 (5·3) |
| 8 | — | — | 15 (5·2) | 21 (5·2) | 25 (5·1) |

* Percentage yield based on glycine.

† In brackets, initial pH of mixture containing 3 mmoles glycine diluted to 21 ml with water.

‡ Sodium bisulphite equivalent of sodium metabisulphite added.

results of experiments carried out at pH 4·7 and 3·5 are shown in Table 2. The reaction at pH 4·7 followed a first order course up to 6, 10, and 12 per cent. yield at 25, 37·5, and 49·5 °C and to at least 15 per cent. at 75 °C. The curves then began to flatten although the reaction mixtures were colourless and the pH was unchanged. Rate constants (Table 2) were calculated from points which fell on the first order curve and the reaction rate at pH 4·7 was 1·6–1·7 times that at pH 3·5. The reaction was also studied at pH 5·6 and in this case the flattening was more pronounced. Rate constants were not calculated but comparison of the curves showed that the initial rate at pH 5·6 was 2·1–2·4 times that at pH 4·7. The reaction mixtures with pH 4·7 and 5·6 resulted from the use of different samples of sodium metabisulphite. It was presumed that the sample which gave the higher pH contained some sodium carbonate

and it was shown that the yield of deoxyglycinofructose was increased when the pH was raised by the addition of a small quantity of this salt. Lea and Hannan (1949) found that the initial rate of loss of free amino nitrogen in a casein–glucose mixture increased with increasing pH.

The rate constants shown in Table 2 were used for the appropriate plots for the Arrhenius equation and the equation derived from the theory of absolute reaction rates (Glasstone, Laidler, and Eyring 1941). There was a good fit in

TABLE 2

SOME RATE CONSTANTS FOR THE REACTION OF GLYCINE AND GLUCOSE IN THE PRESENCE OF BISULPHITE

| Reaction Mixture | Rate Constants | | Activation Energy (kcal/mole) | Entropy of Activation (E.U.) |
|---|---|---|---|---|
| | Temperature (°C) | $k_{obs.}$ ($sec^{-1}$) | | |
| Glycine (1 mole), glucose (4 moles), sorbitol (3·7 moles), sodium bisulphite (1 mole), water 23·8%, pH 4·7* | 25 | $1·39 \times 10^{-8}$ | $E_{obs.} = 26·0$ $PZ = 1·13 \times 10^{11}$ | $\Delta S‡$ at 50 °C $= -10·2$ |
| | 37·5 | $7·53 \times 10^{-8}$ | | |
| | 49·5 | $3·81 \times 10^{-7}$ | | |
| | 75 | $7·92 \times 10^{-6}$ | | |
| | 100 | $1·13 \times 10^{-4}$ | | |
| As above plus hydrochloric acid (0·11 mole), water 23·6%, pH 3·5* | 37·5 | $4·64 \times 10^{-8}$ | $E_{obs.} = 26·5$ $PZ = 1·59 \times 10^{11}$ | $\Delta S‡$ at 50 °C $= -9·5$ |
| | 49·5 | $2·28 \times 10^{-7}$ | | |
| | 63 | $1·21 \times 10^{-6}$ | | |
| | 75 | $4·62 \times 10^{-6}$ | | |

* pH of mixture containing 3 mmoles glycine with 25 ml water added.

both cases over the whole range and all results were used to calculate the activation energy and entropy of activation (Table 2). The results for the two pH levels were not significantly different. Lea and Hannan (1949) derived an activation energy of 29·0 kcal over the range 15 to 70 °C from the rate of loss of free amino groups in a mixture of casein and glucose. They considered that the value was probably constant over the range 0 to 90 °C.

### III. Effect of Phosphate and Malate

The apricots previously studied contained 50 moles of malic acid and 10 moles of citric acid to 11 moles of amino acid (Anet and Reynolds 1957) and the pH of the fruit was 3·5. The effect of some acids and salts on the rate of reaction of glycine and glucose at 49·5 °C and pH 3·5 was studied by adding small quantities of concentrated acid or salt solutions to the glycine–glucose–bisulphite–sorbitol mixture shown in Table 2. The shape of the curves was the same as before and pseudo first-order rate constants were calculated from suitable points. Acetic acid (1·06 moles) increased the reaction rate by a factor of 2·4. With phosphoric acid (0·05 mole with 0·97 mole of sodium dihydrogen phosphate) the factor was 3·2 and with malic acid (0·47 mole with 0·52 mole of potassium hydrogen malate) 2·9. With 0·2 mole of sodium dihydrogen phosphate the factor was 1·6.

The 1 : 8 : 1 (glycine : glucose : bisulphite) mixture was used to study in more detail the effect of adding different proportions of salts. Values obtained without bisulphite were included when it was found that the buffering prevented any change in pH, over the part of the curve being studied, even though the mixtures became brown. The curves had the same shape with or without bisulphite and, as the reaction rate increased, were first order to higher yields, points up to 44 per cent. being used to calculate the highest rate constant obtained. The results are shown in Table 3. Reaction rates in the presence of phosphate alone were 1·2–1·3 times those for phosphate plus bisulphite. The plot of $k$ against moles of phosphate, with or without bisulphite, was a curve with pronounced flattening in the region of 1 mole of phosphate per mole of glycine. The backward extension of both curves intersected the $k$ axis at approximately $4 \cdot 7 \times 10^{-7}$ sec$^{-1}$. The salt effect with sodium chloride was investigated and found to be fairly small (Table 3).

The effect of adding malic acid at pH 3·5 was studied with a constant ratio of malic acid to hydrogen malate ion, with the addition of potassium chloride to maintain a constant ionic strength. The rate constants (Table 3) plotted against moles of malic acid or malate gave curves which flattened as the concentration increased. However, the points fell precisely on straight lines when $k$ was plotted against the square root of the concentration. These lines passed through the origin. The rate constants for phosphate without bisulphite were plotted in the same way and found to fit a straight line with a maximum divergence at any one point of $0 \cdot 7 \times 10^{-7}$; the line intersected the $k$ axis at $5 \cdot 3 \times 10^{-7}$. The experimental techniques did not permit the extension of the concentration curves to higher levels. It seems possible that the concentration effects at the lower levels are complicated by the glycine and deoxyglycinofructose both of which could act as acid-base catalysts. Further investigation was deferred until methods more suitable for detailed studies could be developed.

The nature of the catalytic effect exerted by the dihydrogen phosphate and hydrogen malate ions cannot be determined from the above results. It is, however, likely that they act as both acid and base catalysts. Recent work by Rosen, Woods, and Pigman (1958) has shown that the Amadori rearrangement, carried out in methanol at 100 °C, is almost certainly subject to general acid-base catalysis. Although these workers used a preformed glucosylamine their results would in many cases be directly applicable to the reaction of an amine and glucose, but in systems at pH 3·5–3·6, as in the present work, the rate of formation of the glucosylamine may be important and is likely to be acid-catalysed.

The freeze-dried fruit contained sugar half-esters of malic acid (Anet and Reynolds 1957) and the glucose esters present have now been shown (Ingles and Reynolds 1959) to be glucose-1- and glucose-6-hydrogen malates. Ester formation could influence the rate of formation of deoxyglycinofructose in various ways. However, it was shown, by the determination of free inorganic phosphate, that esterification did not occur in the phosphate mixtures. A malate mixture lost only 2 per cent. of free malate in 15 days at 49·5 °C, whereas mixtures were heated for no more than 3 days for the determination of rate constants.

TABLE 3

RATE CONSTANTS FOR THE REACTION OF GLYCINE (1 MOLE) AND GLUCOSE (8 MOLES) AT 49·5 °C IN THE PRESENCE OF SOME ACIDS AND SALTS

| Acids and Salts Added (mole/mole glycine) | Without Sodium Bisulphite | | | With Sodium Bisulphite (1 mole/mole glycine) | | |
|---|---|---|---|---|---|---|
| | Water (% calc.) | pH* | $k_{obs.}$ ($10^7$ $sec^{-1}$) | Water (% calc.) | pH* | $k_{obs.}$ ($10^7$ $sec^{-1}$) |
| $H_3PO_4$ 0·08, $NaH_2PO_4$ 0·42† .. .. .. | 22·7 | 3·6 | 10·6 | 21·3 | 3·7 | 8·9 |
| „ 0·08, „ 0·92 .. .. .. | 22·6 | 3·6 | 14·5 | 21·2 | 3·7 | 12·3 |
| „ 0·13, „ 2·00 .. .. .. | 21·8 | 3·5 | 17·1 | 20·5 | 3·6 | 14·9 |
| „ 0·09, „ 3·64 .. .. .. | 21·7 | 3·6 | 21·9 | 20·5 | 3·7 | 16·4 |
| HCl 0·05 .. .. .. .. .. .. | 24·4 | 3·67 | 4·75 | — | — | — |
| „ 0·05, NaCl 1·04 .. .. .. .. | 23·7 | 3·66 | 4·49 | — | — | — |
| „ 0·05, „ 2·08 .. .. .. .. | 23·6 | 3·67 | 4·13 | — | — | — |
| „ 0·05, „ 2·96 .. .. .. .. | 23·7 | 3·66 | 3·89 | — | — | — |
| Malic acid 0·261,‡ KH malate 0·239, KCl 2·261 | 24·1 | 3·51 | 8·53 | — | — | — |
| „ „ 0·442, „ „ 0·558, „ 1·942 | 24·3 | 3·51 | 12·06 | — | — | — |
| „ „ 0·623, „ „ 0·877, „ 1·623 | 24·5 | 3·51 | 14·91 | — | — | — |
| „ „ 0·804, „ „ 1·196, „ 1·304 | 24·6 | 3·51 | 17·29 | — | — | — |

* pH of mixture containing 3 mmoles glycine diluted to 100 ml with water.

† The values for moles of dihydrogen phosphate used in plots against $k$ were 0·48, 0·98, 2·08, 3·70; compare malate values below.

‡ Ratio of malic acid to potassium hydrogen malate to give pH 3·51 without glycine was 0·181 : 0·319; 1 mole of glycine reduced the potassium hydroxide required by 0·08 mole. The values in the table are those used to prepare the reaction mixtures. The values plotted against $k$ were 0·181, 0·362, 0·543, 0·724 for malic acid and 0·319, 0·638, 0·957, 1·276 for hydrogen malate ion.

## IV. Relation of Results to Non-Enzymic Browning in Dried Fruit

Some interesting relationships can be seen between the above results and some of the effects found in studies of non-enzymic browning in fruit. From the rate of browning of sulphured dried apricots at temperatures between 22 and 49 °C Stadtman *et al.* (1946) derived an apparent activation energy of 26 kcal, the same as that now found for the reaction of glycine and glucose, in the presence of bisulphite, for 25 to 100 °C. It can be deduced from the rate constants now determined that the treatment of fruit with sulphur dioxide would be expected to reduce the rate of reaction of amino acids and glucose by less than 30 per cent. This could be important but the degree of inhibition of browning by bisulphite suggests that other mechanisms are also involved.

Haas and Stadtman (1949) found that the addition of the anionic fraction of the soluble constituents of apricots enhanced the browning of the mixed cationic and neutral fractions. This can be interpreted as resulting from the catalytic effect of the organic acids on the rate of reaction of the amino acids and glucose. Extrapolation of the line obtained for malate above indicates that the organic acids in the fruit would increase the rate of reaction about seven times.

## V. Experimental

(*a*) *Preparation of Reaction Mixtures.*—Anhydrous glucose, or a glucose–sorbitol mixture, was heated in a stoppered flask or test tube in a boiling water-bath with sufficient water, dilute acid, or buffer solution to give a final water content, after the addition of glycine and sodium metabisulphite, of 10–25%. Heating for 1–10 min (depending on the total quantity) gave a clear syrup. Glycine and sodium metabisulphite were added and dissolved rapidly in the hot syrup by shaking. The mixture was either heated at 100 °C or cooled rapidly to room temperature and then brought to the required temperature. At the end of the reaction time the syrup was dissolved in water (*c.* 4 vol., or 10 vol. for small-scale experiments) and ethanol (95%) was added to give a 70% ethanolic solution.

(*b*) *Chromatography.*—The 70% ethanolic solution of reaction products was run through a column of " Dowex 50–X4 " (H form, equilibrated in deaerated 70% ethanol). The column was washed with 70% ethanol and 2 or 3 smaller columns (H form, in water) were attached. The bases were displaced with 0·1N aqueous ammonia. The fractions collected were examined on paper chromatograms run in *n*-butanol–acetic acid–water (4 : 1 : 1 v/v) and treated with ninhydrin, silver nitrate–sodium hydroxide, and triphenyltetrazolium chloride–sodium hydroxide in the cold (cf. Anet and Reynolds 1957).

(*c*) *Determination of Reducing Value of Basic Reaction Products.*—Syrupy reaction mixtures originally containing 3 mmoles glycine were (i) dissolved in water (40 ml) and 95% ethanol (110 ml) was added, or (ii) made up to 50 or 100 ml with water and an aliquot diluted with water and 95% ethanol to give 150 ml 70% ethanol. The solution was run through a column of " Dowex 50–X4 " (5 g ; H form, equilibrated in deaerated 70% ethanol) and the column was washed with 70% ethanol (100 ml) and water (50 ml) until free from glucose and sulphurous acid. The bases were recovered from the column by washing with (1) 0·2N sodium hydroxide (95–110 ml) and water to give 250 ml, or (2) N hydrochloric acid (50 ml) and water to give 250 ml or 100 ml. Aliquots of 1–10 ml were taken for analysis.

The reducing value was determined by the ferricyanide–arsenomolybdate method described by Ting (1956) for the determination of reducing sugars, with the following modifications : (i) the proportions of sodium carbonate and disodium phosphate in the ferricyanide reagent were halved to avoid crystallization, (ii) 10 ml of this reagent was used, (iii) the sample was heated with ferricyanide in a ¾ in. test tube and the mixture transferred to a 100 ml volumetric flask, (iv) tubes were heated for 10 min (same result for 2–12 min) in a boiling water-bath, (v) 8 ml of the arseno-

molybdate reagent was added. The colour yield for pure 1-deoxy-1-glycinofructose was similar to that recorded by Ting (1956) for glucose and fructose. The test solutions were compared with a standard solution of deoxyglycinofructose run at the same time, as the colour yield varied slightly from day to day.

(*d*) *Products of the Glucose–Glycine–Bisulphite Reaction.*—(i) *At 100 °C.* Glucose (240 g), water (34 ml), glycine (24 g), and sodium metabisulphite (32 g) were heated on a boiling water-bath for 1 hr, giving a golden-brown syrup, and the products were studied by chromatography. The fractions (15 ml) collected contained deoxyglycinofructose, glycine, and unknowns as follows: 1–6, deoxyglycinofructose with smaller quantities of U1–U3; 7–88, deoxyglycinofructose with faint traces of impurities; 89–100, deoxyglycinofructose with small amounts of U4–U6, glycine in the later fractions; 101, glycine. Fractions 7–88 were evaporated under reduced pressure to *c.* 150 ml. Ethanol (95%; *c.* 600 ml) was added gradually with warming. Almost colourless deoxyglycinofructose (23·3 g) crystallized on cooling and a second crop (2·5 g) was obtained when 95% ethanol was added to the filtrate (yield 24·8 g, 33%). One recrystallization from aqueous ethanol gave pure deoxyglycinofructose with the properties previously described (Anet 1957).

All the by-products (U1–U6) reacted on paper chromatograms with ninhydrin, all except U3 reacted with silver nitrate–sodium hydroxide, and U1, U2, U5, and U6 reacted strongly with triphenyl tetrazolium chloride–sodium hydroxide in the cold. U1 appeared to be the main by-product, with lesser amounts of U5 and U6 and traces of U2–U4. The $R_{glucose}$ values of U1–U6 on paper chromatograms run in *n*-butanol–acetic acid–water (4 : 1 : 1 v/v) were 0·26, 0·36, 0·83, 0·36, 0·67, and 0·79.

(ii) *Products Formed at Different Temperatures.* Glucose (17·3 g), sorbitol syrup (23·4 g; water content 29·8%), water (5·3 ml), glycine (1·8 g), and sodium metabisulphite (2·3 g) were held at (1) 100 °C for 5 min, (2) 75 °C for 1 hr, (3) 50 °C for 16 hr, (4) 25 °C for 19 and 118 days. The colourless syrups obtained were studied by chromatography. The chromatograms were similar for all temperatures and showed deoxyglycinofructose with small quantities of the main by-products, U1, U5, and U6. Traces of U2 were detected in (2) and (4).

(iii) *Products Formed in the Presence of Malate and Phosphate.* Glucose (43·2 g; 8 moles), malate buffer (16 ml; 1 mole malic acid, 0·7 mole potassium hydroxide), glycine (2·25 g; 1 mole), and sodium metabisulphite (2·9 g; 1 mole sodium bisulphite) were heated on a boiling water-bath for 1 hr. A similar mixture containing phosphoric acid (1 mole) and sodium hydroxide (0·9 mole) was heated under the same conditions. The products were studied by chromatography. The chromatograms were similar to those obtained without malate or phosphate.

(*e*) *Effect of Proportions of Reactants on Yield of 1-Deoxy-1-glycinofructose.*—Glucose (0·54–8·64 g; 1–16 moles), water (0·15–1·24 ml; 10% by weight of the reaction mixture), glycine (0·225 g; 1 mole), and sodium metabisulphite (0·073–2·32 g; equivalent to 0·25–8 moles sodium bisulphite) were held at 100 °C for 1 hr. The final colour was pale yellow with the higher sulphite concentrations and was translucent reddish brown with the lowest sulphite level. The colour was paler with increasing amounts of glucose at any one sulphite level. The reducing value of the basic products was determined and expressed as percentage yield of deoxyglycinofructose. The results are shown in Table 1. The pH of the unheated reaction mixtures (Table 1) was determined after adding water to bring the volume to 21 ml.

(*f*) *Effect of Heating Time at 100 °C on Yield of Deoxyglycinofructose.*—Glucose (8·64 g; 16 moles), glycine (1 mole), sodium bisulphite (2 moles), and water (10%) were held at 100 °C for 1, 2, and 3 hr (syrups were brownish yellow to reddish brown). The yields were 39, 42, and 35%. Glucose (16 moles), glycine (1 mole), sodium bisulphite (0·5 mole), and water (10%) were held at 100 °C for 30 min and then sodium bisulphite (0·5 mole) was added every 30 min. Heating times were 2 (orange-yellow syrup), 4, and 6 hr (reddish brown syrup) and the yields were 41, 29, and 17%.

(*g*) *Effect of Water Content of Reaction Mixture on Yield of Deoxyglycinofructose at 100 °C.*—Glucose (2·16 g; 4 moles), glycine (1 mole), and sodium bisulphite (2 moles) were heated for 1 hr at 100 °C with 10, 20, and 30% water. The yields were 36, 32, and 27%. Glucose (4·32 g; 8 moles), glycine (1 mole), and sodium bisulphite (1 mole) were heated for 1 hr at 100 °C with

10, 18, 22, 25, and 45% water. The yields were 53, 40, 41, 34, and 26%. The heated reaction mixtures were paler in colour with the higher water contents.

(*h*) *Effect of pH and Temperature on Yield of Deoxyglycinofructose.*—(i) *pH 4·7 at 25, 37·5, 49·5, 75, and 100 °C.* Reaction mixtures containing glucose (2·16 g; 4 moles), sorbitol syrup (2·30 ml; *d* 1·25, water content 29·8%; 3·7 moles sorbitol), water (0·64 ml), glycine (0·225 g; 1 mole), and sodium metabisulphite (0·29 g; 1 mole sodium bisulphite) were prepared in ⅝ in. tubes. The water content of the mixture (calc.) was 23·6%; the pH in 25 ml water was 4·7. The tubes were sealed and stored at the above temperatures for periods varying from 100 days at 25 °C to 20 min at 100 °C. At intervals during that period duplicate tubes were withdrawn and the percentage yield of deoxyglycinofructose determined. The yield at zero time was 0·06%. The points for log (100 —% deoxyglycinofructose) plotted against time fell on a straight line up to 6% yield at 25 °C, 10% at 37·5 °C, and 12% at 49·5 °C. The yields with longer heating times (2 points at 25 °C, 3 at 37·5 °C, 6 at 49·5 °C) were increasingly lower than those required to fit the first order curve. At 75 and 100 °C the last points determined (15 and 13%) fell on the curve. The heated reaction mixtures were colourless except for those held 20 min at 100 °C, which were pale yellow. Pseudo first-order constants were calculated (in $sec^{-1}$, natural logarithms) from points which fell on the curve. The mean values are shown in Table 2. Both log $k$ and log $k/T°A$ gave a straight line when plotted against $1/T°A$. The activation energy ($E_{obs.}$) and probability factor were calculated from the constants for the line derived from the individual values of $k$. $E_{obs.}$ was used to calculate the entropy of activation. The results are shown in Table 2.

(ii) *pH 3·5 at 37·5, 49·5, 63, and 75 °C.* Reaction mixtures were the same as in Section V (*h*) (i) except that 0·5N hydrochloric acid (0·64 ml; 0·11 mole) was used in place of water (0·64 ml). The pH (in 25 ml water) was 3·5 and the water content (calc.) 23·6%. Rate constants were calculated as before. The results are shown in Table 2. At 49·5 °C points for yields up to 11% fell on the first-order curve and then the yield fell off with time. At other temperatures only the early part of the curve was investigated. All reaction mixtures were colourless.

(iii) *pH 5·6 at 25, 37·5, 49·5, 63, and 75 °C.* In earlier experiments reaction mixtures prepared as in Section V (*h*) (i) gave pH 5·6 when dissolved in water (25 ml). The yields of deoxyglycinofructose were higher than at pH 4·7 and the yield curves showed a more pronounced flattening. There were insufficient suitable points for the calculation of rate constants. It was estimated from curves that at 25, 37·5, and 49·5 °C the yield at pH 5·6 was 2·1–2·4 times that at pH 4·7 over the first half of the time (during this time the reaction at pH 4·7 followed a first order course). At the end of the time studied the yield at pH 5·6 was 1·5–1·8 times that at pH 4·7; at this stage some of the mixtures initially at pH 5·6 were pale yellow and the pH was 5·3.

Reaction mixtures giving pH 5·3 and 5·8 were prepared with sodium carbonate solution (0·64 ml, 0·1N and 0·33N) in place of water (0·64 ml), using the sodium metabisulphite which gave pH 4·7 with water. The yields for pH 4·7, 5·3, and 5·8 after 40 hr at 50 °C were 4·9, 7·6, and 14·7%, and after 64 hr 7·5, 11·4, and 16·7%.

(*i*) *Effect of Added Acids and Salts on Yield of Deoxyglycinofructose.*—(i) *Mixtures Containing Sorbitol and Bisulphite.* Reaction mixtures were the same as in Section V (*h*) (i) except the added water (0·64 ml) was replaced by a similar volume of acid or buffer solution. The pH of the mixtures with water (25 ml) added was 3·5. Tubes were held at 49·5 °C and pseudo first-order constants calculated as before. The moles of added acid and salt per mole of glycine, the values for $10^7 k_{obs.}(sec^{-1})$, and the water contents of the mixtures were as follows: acetic acid (1·06 moles), $k$ 5·50, water 23·8%; sodium acetate (0·85), hydrochloric acid (0·86), $k$ 5·10, water 20·5%; malic acid (0·10). $k$ 3·23, water 23·1%; malic acid (0·47), potassium hydrogen malate (0·52), $k$ 6·70, water 23·0%; hydrochloric acid (0·09), sodium dihydrogen phosphate (0·21), $k$ 3·67, water 23·5%; hydrochloric acid (0·08), sodium dihydrogen phosphate (0·85), $k$ 6·98, water 21·5%; phosphoric acid (0·05), sodium dihydrogen phosphate (0·97), $k$ 7·24, water 23·7%.

(ii) *Mixtures Without Sorbitol.* Reaction mixtures were prepared with glucose (4·32 g; 8 moles), glycine (0·225 g; 1 mole), and acid or buffer mixtures to give the composition shown in Table 3. Where sodium metabisulphite was added the quantity was 0·29 g (1 mole sodium

bisulphite). The mixture containing 2·96 moles sodium chloride was supersaturated and a small quantity of sodium chloride crystallized out during the period of reaction. The two stronger malate buffers were supersaturated and were added to the glucose immediately after being brought to room temperature; there was no crystallization of salt from the reaction mixture. The tubes were heated at 49·5 °C. The reaction mixtures were dissolved in water and made up to 100 ml to determine the pH (Table 3). The mixtures containing bisulphite remained colourless. The others slowly turned brown. Rate constants (Table 3) were determined as before. With hydrochloric acid and sodium chloride the reaction rate had decreased slightly when the yield was 12–15% (depending on the chloride content). With the highest level of phosphate the reaction followed a first order course to 44% yield without bisulphite and to 37% with bisulphite.

(*j*) *Determination of Inorganic Phosphate in Glucose–Glycine–Bisulphite–Phosphate Mixtures.*—Reaction mixtures containing glucose (8 moles), glycine (1 mole), sodium bisulphite (1 mole), phosphoric acid (1 mole), and sodium hydroxide (0·92 mole) (cf. Table 3) were held at 49·5 °C for 3 to 15 days together with a corresponding set of tubes containing no glycine. The syrups were dissolved in water and free inorganic phosphate was determined by the method of Allen (1940). All samples gave the same value as the unheated mixture. A reaction mixture containing 3·64 moles phosphate per mole of glycine (see Table 3) showed no loss in free phosphate after 3 days at 49·5 °C.

(*k*) *Determination of Malic Acid in a Glucose–Glycine–Bisulphite–Malate Mixture.*—A syrup containing glucose–glycine–bisulphite–malate (8 : 1 : 1 : 1; pH 3·6) was held for 15 days at 49·5 °C and dissolved in water. The acids present were determined by elution chromatography using a column of " Deacidite FF " in the formate form (Ingles and Reynolds 1959). The malic acid peak was equivalent to 98% of the malic acid used in the mixture. Three small peaks were also present.

## VI. Acknowledgments

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# CHEMISTRY OF NON-ENZYMIC BROWNING

## VI. THE REACTION OF ALDOSES WITH AMINE BISULPHITES

By D. L. INGLES*

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*Summary*

The crystalline glucose-aniline bisulphite addition compound, D-*glycero*-D-*ido*-1-anilino-2,3,4,5,6-pentahydroxyhexyl anilinium sulphonate, was prepared from the reaction of D-glucose with aniline bisulphite in water and in methanol. The D-*glycero*-D-*ido*-1-anilino-2,3,4,5,6-pentahydroxyhexyl piperidinium sulphonate was prepared by adding piperidine bisulphite to preformed *N*-phenyl-D-glucosylamine. Other new compounds of similar structure were prepared from the reaction of aniline bisulphite with D-galactose and D-mannose.

*cyclo*Hexylamine bisulphite reacted with D-glucose to form crystalline D-*glycero*-D-*ido*-1,2,3,4,5,6-hexahydroxyhexyl *cyclo*hexylammonium sulphonate. When treated with aniline this compound formed D-*glycero*-D-*ido*-1-anilino-2,3,4,5,6-pentahydroxyhexyl *cyclo*hexylammonium sulphonate.

The D-*glycero*-D-*ido*-1-anilino-2,3,4,5,6-pentahydroxyhexyl potassium and anilinium sulphonates were interconverted by use of a cation-exchange resin. Acetylation of the anilinium sulphonate in pyridine gave the tetra-acetate of *N*-phenyl-D-glucosylamine.

### I. INTRODUCTION

Previous attempts to prepare crystalline glucose-amine bisulphite addition compounds (Adams and Garber 1949) were unsuccessful although reaction of non-sugar aldehydes with amine bisulphites occurred readily. A previous paper (Ingles 1959*a*) described amine derivatives of glucose potassium sulphonate. The present paper reports the preparation of aldose-amine bisulphite addition compounds.

Solutions of amine bisulphites were prepared by bubbling sulphur dioxide into a mixture of amine and water or into an alcoholic solution of the amine. Amine bisulphites in solution probably contain varied amounts of sulphur dioxide. However, when sulphur dioxide was passed through aniline in water a white crystalline solid formed analysing approximately for $(C_6H_5NH_2)_2H_2SO_3$. This solid dissolved in water and formed a syrup when treated with additional sulphur dioxide.

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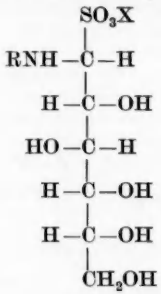
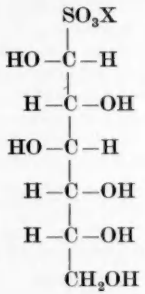
Aniline bisulphite in aqueous solution reacted with D-glucose in water to form a white crystalline compound I (R=-phenyl, X=anilinium). Crystal-



(I)



(II)

lization began within 15 min of mixing the reactants and was 98·0 per cent. complete after 16 hr at room temperature. D-Galactose behaved similarly. With D-mannose crystallization was even more rapid, an almost quantitative yield of product forming in 10 min. The galactose-aniline bisulphite addition compound crystallized with one molecule of water of crystallization whereas the glucose and mannose compounds contained no water of crystallization. The glucose-aniline bisulphite compound I (R=-phenyl, X=anilinium) is named D-*glycero*-D-*ido*-1-anilino-2,3,4,5,6-pentahydroxyhexyl anilinium sulphonate (Rules of Carbohydrate Nomenclature 1952, rules 6 and 22). Evidence supporting the configuration of $C_1$ of this and other similar compounds discussed here will be presented in a subsequent paper. The glucose-aniline bisulphite addition compound was also prepared in methanol solution. A methanol solution of aniline bisulphite was refluxed with D-glucose until the sugar dissolved, the crystalline product being obtained by cooling the resultant solution.

*cyclo*Hexylamine bisulphite reacted with D-glucose in water to form the crystalline addition compound II (X=*cyclo*hexylammonium). This compound contained only one *cyclo*hexylamine moiety and its structure was unlike the aniline bisulphite addition compound of glucose. The compound was similar in structure to the aldose potassium sulphonates previously described (Ingles 1959*a*) and to the amine bisulphite addition compounds of non-sugar aldehydes described by Adams and Lipscomb (1949) and Adams and Garber (1949). Recrystallization was effected from methanol in which solvent the compound dissolved after refluxing for 15 min.

The mechanism by which I is formed is not definitely known. Adams and Garber (1949) formulated the reaction of a non-sugar aldehyde with an amine bisulphite to form the aldehyde-amine bisulphite addition compound as

$$RCHO + R'NH_2.H_2SO_3 \rightarrow R.CHOH.SO_3NH_3R'.$$

Compound I may form by reaction of a compound such as II (X=anilinium) with a molecule of aniline. Alternatively *N*-phenyl-D-glucosylamine may form first and add on a molecule of aniline bisulphite. Evidence for both these mechanisms has been obtained.

When heated with aniline at 100 °C, II (X=*cyclo*hexylammonium) formed a homogeneous syrup. After extraction with ether to remove excess aniline the residual syrup crystallized, forming I (R=-phenyl, X=*cyclo*hexylammonium).

However, the compound I (R=-phenyl, X=piperidinium) was also formed by adding a methanol solution of piperidine bisulphite to preformed *N*-phenyl-D-glucosylamine in methanol at 40 °C. On cooling, the product crystallized. The alternative mechanism is therefore also possible. Thus when an aldose reacts to form compounds of type I, both mechanisms may be operative, since addition compounds such as II and glycosylamines (cf. Weygand 1939) form in aqueous solution.

The relationship of I (R=-phenyl, X=anilinium) to the aniline derivative of glucose potassium sulphonate (Ingles 1959*a*) was shown by the interconversion of the two compounds. A solution of I (R=-phenyl, X=anilinium) in 75 per cent. aqueous methanol gave I (R=-phenyl, X=potassium) when treated with "Dowex 50" resin, potassium form. Similarly, when the aniline derivative of glucose potassium sulphonate, obtained as a syrup by heating glucose potassium sulphonate with aniline, was dissolved in 75 per cent. aqueous methanol and treated with "Dowex 50" resin, aniline form, I (R=-phenyl, X=anilinium) was isolated. These exchange reactions confirm the acyclic structure assigned to these compounds. Of the two aniline groups in I (R=-phenyl, X=anilinium), one is ionic in character and exchanged with potassium ions adsorbed on a cation resin. The other group is bound to the aldose as in *N*-phenyl-D-glucosylamine. The tetra-acetate of *N*-phenyl-D-glucosylamine was isolated when compound I was acetylated with acetic anhydride in pyridine, the sulphonate group being removed by the basic pyridine as it is by aqueous sodium hydroxide (Ingles 1959*b*).

## II. Experimental

Melting points are uncorrected. Microanalyses were carried out by the C.S.I.R.O. Microanalytical Laboratory at the University of Melbourne.

(*a*) *Preparation of Amine Bisulphites.*—The following general procedures were adopted for the preparation of the amine bisulphites used in the reactions described below. Sulphur dioxide was bubbled through a suspension or solution of the amine in water. Heat was evolved and a white crystalline solid usually appeared. Further sulphur dioxide was bubbled into the reaction flask until a clear homogeneous solution was obtained and until no further exothermic heat was produced. This solution was then used directly. An alternative procedure involved bubbling sulphur dioxide into a methanol solution of the amine until the initial exothermic absorption reaction was complete. This methanol solution of the amine bisulphite was also used immediately in further reactions.

(*b*) *Reaction of Aldoses with Aniline Bisulphite.*—(i) *Reaction of Glucose.* (1) D-Glucose (100 g) dissolved in water (150 ml) was treated at room temperature with a solution of aniline bisulphite formed from aniline (130 ml) in water (300 ml). Crystallization began within ½ hr and was almost complete in 16 hr. The product, D-glycero-D-ido-*1-anilino-2,3,4,5,6-pentahydroxyhexyl anilinium sulphonate*, was filtered and washed with ethanol. The yield of product was 235 g (98·0%), m.p. 114 °C (decomp.), $[\alpha]_D^{25}$ −7·5° (5 min)→17·6° (const. 30 min, *c*, 3·4, 10% acetic acid) (Found: C, 50·3; H, 6·2; O, 30·0; N, 6·0; S, 6·9%. Calc. for $C_{18}H_{26}O_8N_2S$: C, 50·1; H, 6·0; O, 29·8; N, 6·5; S, 7·4%).

(2) D-Glucose (20 g) was refluxed for 10 min with a solution of aniline bisulphite formed from aniline (20 ml) dissolved in methanol (30 ml). The solution darkened on heating and was filtered

to remove the last traces of undissolved glucose. After standing overnight at +1 °C the crystalline product was filtered and washed with ethanol until white. The yield was 25·2 g (52·2%), m.p. 113 °C (decomp.), $[\alpha]_D^{25}$ —7·0° (6 min)→17·5° (const. 30 min, *c*, 3, 10% acetic acid) (Found : C, 50·6 ; H, 6·0 ; O, 30·3 ; N, 6·5 ; S, 7·0%. Calc. for $C_{18}H_{26}O_8N_2S$ : C, 50·1 ; H, 6·0 ; O, 29·8 ; N, 6·5 ; S, 7·4%).

(ii) *Reaction of Galactose.* D-Galactose (40 g) dissolved in water (40 ml) was treated at room temperature with aniline bisulphite formed from aniline (50 ml) in water (50 ml). After standing overnight the crystalline product, D-glycero-L-gluco-*1-anilino-2,3,4,5,6-pentahydroxyhexyl anilinium sulphonate* (80 g, 89·9%), was filtered and washed with ethanol. The m.p. was 81·0 °C (decomp.), $[\alpha]_D^{25}$ 11·0° (2 min)→28·6° (const. 15 min, *c*, 1·6, 10% acetic acid) (Found : C, 48·8 ; H, 6·1 ; N, 6·0 ; O, 32·2 ; S, 6·7%. Calc. for $C_{18}H_{26}O_8N_2S.H_2O$ : C, 48·3 ; H, 6·2 ; N, 6·3 ; O, 32·3 ; S, 7·1%).

(iii) *Reaction of Mannose.* D-Mannose (5 g) dissolved in water (5 ml) was treated at room temperature with a solution of aniline bisulphite formed from aniline (16 ml) in water (30 ml). Crystallization began immediately and after 10 min the product, D-glycero-D-galacto-*1-anilino-2,3,4,5,6-pentahydroxyhexyl anilinium sulphonate*, was filtered and washed with ethanol. The yield of product was 10 g (83%), m.p. 141 °C (decomp.), $[\alpha]_D^{25}$ 32·0° (7 min)→14·3° (const. 5 hr, *c*, 2·0, 10% acetic acid) (Found : C, 50·3 ; H, 6·1 ; O, 29·6 ; N, 6·5 ; S, 7·4%. Calc. for $C_{18}H_{26}O_8N_2S$ : C, 50·1 ; H, 6·0 ; O, 29·8 ; N, 6·5 ; S, 7·4%).

(*c*) *Reaction of N-Phenyl-D-glucosylamine with Piperidine Bisulphite.*—D-Glucose (18 g) was refluxed with aniline (9·3 ml) in methanol (25 ml) for 2 hr to yield a clear solution of *N*-phenyl-D-glucosylamine. A solution of piperidine bisulphite, formed from piperidine (8·5 ml) in methanol (25 ml) and water (1 ml), was added and the mixed solutions warmed to 40 °C and then transferred to +1 °C for 16 hr. The crystalline product, D-glycero-D-ido-*1-anilino-2,3,4,5,6-pentahydroxyhexyl piperidinium sulphonate*, was filtered and washed with ethanol. The yield was 15·0 g (36·0%), m.p. 99 °C (decomp.), $[\alpha]_D^{25}$ —37·5° (1 min)→21·6° (const. 15 min, *c*, 1·2, 10% acetic acid) (Found : C, 49·6 ; H, 6·3 ; O, 30·6 ; N, 6·3 ; S, 6·9%. Calc. for $C_{17}H_{28}O_8N_2S$ : C, 49·0 ; H, 6·0 ; O, 30·6 ; N, 6·7 ; S, 7·5%).

(*d*) *Reaction of Glucose with* cyclo*Hexylammonium Bisulphite.*—D-Glucose (28 g) dissolved in water (30 ml) was treated at room temperature with a solution of *cyclo*hexylammonium bisulphite formed from *cyclo*hexylamine (15 ml) in water (20 ml). After standing at +1 °C for 2 days the crystalline product, D-glycero-D-ido-*1,2,3,4,5,6-hexahydroxyhexyl* cyclo*hexylammonium sulphonate* was filtered and washed with ethanol. The yield was 14·3 g (25·5%), m.p. 107 °C (decomp.), $[\alpha]_D^{25}$ —7·7° (initial) (*c*, 1·7, 10% acetic acid) (Found : C, 40·1 ; H, 7·4 ; O, 40·4 ; N, 3·6 ; S, 8·8%. Calc. for $C_{12}H_{23}O_9NS$ : C, 39·8 ; H, 7·7 ; O, 39·8 ; N, 3·9 ; S, 8·8%).

(*e*) *Reaction of the Glucose* cyclo*Hexylammonium Bisulphite Addition Compound with Aniline.*—The product of the reaction (*d*) next above (5 g) was heated with aniline (3 ml) at 100 °C for 10 min when a homogeneous syrup resulted. Excess aniline was extracted with ether. The residual syrup crystallized after 3 days at room temperature. The crystals were stirred with ethanol (20 ml), filtered, and washed with ethanol. The yield of the crystalline product, D-glycero-D-ido-*1-anilino-2,3,4,5,6-pentahydroxyhexyl* cyclo*hexylammonium sulphonate*, was 4·2 g (69·9%), m.p. 101 °C, $[\alpha]_D^{25}$ —15·0° (3 min)→18·7° (const. 2 hr, *c*, 1·2, 10% acetic acid) (Found : C, 48·8 ; H, 7·5 ; O, 30·1 ; N, 6·0 ; S, 7·2%. Calc. for $C_{18}H_{33}O_8N_2S$ : C, 49·4 ; H, 7·5 ; O, 29·5 ; N, 6·4 ; S, 7·3%).

(*f*) *Cation-Exchange Reactions.*—(i) *Reactions with "Dowex 50", Potassium Form.* The D-*glycero*-D-*ido*-1-anilino-2,3,4,5,6-pentahydroxyhexyl anilinium sulphonate (15 g) (prepared according to (*b*) above) was dissolved in 75% aqueous methanol (50 ml) and passed down a column of "Dowex 50" resin, potassium form (30 g). The column was washed with 75% aqueous methanol (50 ml) and the effluent evaporated under vacuum at 40 °C. The syrup obtained crystallized slowly during 3 days at room temperature. The crystals were mixed with ethanol, filtered, and washed with ethanol. The yield of the product, D-*glycero*-D-*ido*-1-anilino-2,3,4,5,6-pentahydroxyhexyl potassium sulphonate, was 6·1 g (44·5%), m.p. 137 °C (decomp.), $[\alpha]_D^{25}$ —14·0°

(3 min)→+22° (const. 30 min, *c*, 2, 10% acetic acid) (Found : C, 35·8 ; H, 5·1 ; N, 3·2 ; S, 8·1 ; K, 9·9%. Calc. for $C_{12}H_{18}O_8NSK.H_2O$ : C, 36·5 ; H, 5·1 ; N, 3·6 ; S, 8·1 ; K, 9·9%) (cf. Ingles 1959*a*).

(ii) *Reaction with " Dowex 50 ", Aniline Form.* The D-*glycero*-D-*ido*-1-anilino-2,3,4,5,6-pentahydroxyhexyl potassium sulphonate (20 g) was prepared as a 20% methanolic solution by the procedure previously described (Ingles 1959*a*). Water (12 ml) was added and the solution passed down a column of " Dowex 50 " resin, aniline form (30 g). The column was washed with 75% aqueous methanol (50 ml) and the effluent evaporated under vacuum at 40 °C. The syrup thus obtained crystallized on standing. The crystals were filtered and washed with ethanol. The yield of the product, D-*glycero*-D-*ido*-1-anilino-2,3,4,5,6-pentahydroxyhexyl anilinium sulphonate, was 5·2 g (23·7%), m.p. 114 °C (decomp.), $[\alpha]_D^{25}$ −7·0 (4 min)→17·6° (const. 30 min, *c*, 3·2, 10% acetic acid) (Found : C, 50·1 ; H, 6·0 ; O, 29·8 ; N, 6·6 ; S, 7·3%. Calc. for $C_{18}H_{26}O_8N_2S$ : C, 50·1 ; H, 6·0 ; O, 29·8 ; N, 6·5 ; S, 7·4%). These values compare with similar data obtained for this compound synthesized directly from glucose and aniline bisulphite (see Section II (*b*)).

(*g*) *Acetylation of* D-Glycero-D-ido-*1-anilino-2,3,4,5,6-pentahydroxyhexyl Anilinium Sulphonate.*—The compound (10 g) was dissolved in pyridine (50 ml) by gentle warming. After cooling to +1 °C, acetic anhydride (25 ml) was added slowly and the mixed solution kept at +1 °C for 24 hr. The reaction mixture was poured into iced water (1 l.) when an oil separated and slowly crystallized. The yield of crude product was 7 g (71·3%). The product, the tetra-acetate of *N*-phenyl-D-glucosylamine, was twice recrystallized from ethanol, m.p. 140 °C, $[\alpha]_D^{25}$ 42·0° (final) (*c*, 3·4 in chloroform). Frèrejacque (1937) has found $[\alpha]_D^{25}$ 41·6° (Found : C, 56·8 ; H, 6·0 ; O, 34·0 ; N, 3·3%. Calc. for $C_{20}H_{25}O_9N$ : C, 56·8 ; H, 5·9 ; O, 34·0 ; N, 3·3%).

## III. Acknowledgment

The author is indebted to Dr. T. M. Reynolds for helpful discussion.

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# CHEMISTRY OF NON-ENZYMIC BROWNING

## VII. CRYSTALLINE DI-D-FRUCTOSE-GLYCINE AND SOME RELATED COMPOUNDS

By E. F. L. J. Anet*

[*Manuscript received November* 28, 1958]

*Summary*

Glycine and D-glucose react to give *N*-(carboxymethyl)-1-amino-1-deoxy-D-fructose. The further reaction with D-glucose or D-mannose to yield di-D-fructose-glycine [*NN*-di-(D-*arabino*-3,4,5,6-tetrahydroxy-2-oxohexyl)glycine] is now reported. Di-D-fructose-glycine was isolated as the crystalline dihydrate, m.p. 112 °C, $[\alpha]_D^{25}$ —78°, and its structure was deduced from its formation, its decomposition, and from the products formed on periodate oxidation. Infra-red spectra showed that both the above compounds occurred in a ring form, probably the β-D-fructopyranose form. Di-D-fructose-glycine was a labile compound which decomposed to give a quantitative yield of *N*-(carboxymethyl)-1-amino-1-deoxy-D-fructose and unknown carbonyl compounds. The rate of this decomposition has been studied at various pH values and temperatures.

Other primary aliphatic amines and aldoses gave the corresponding diketose-amines which have similar properties. These compounds possibly have an important role in non-enzymic browning reactions.

### I. Introduction

Glycine reacts rapidly and reversibly with D-glucose to give the glycosylamine derivative (Micheel and Klemer 1952) which undergoes slowly and irreversibly the Amadori rearrangement to give D-fructose-glycine† (I ; Abrams, Lowy, and Borsook 1955 ; Richards 1956 ; Anet 1957 ; Dubourg and Devillers 1957). These are typical reactions of amines with aldoses and are generally accepted as being the first steps in the " Maillard reaction " or non-enzymic browning ; the nature of subsequent reactions is still obscure (Hurd and Buess 1956). However, in the case of an aminodeoxyketose derived from a primary amine, the nitrogen is secondary and should react with one more molecule of aldose in the above way. Thus glycine and D-glucose should yield di-D-fructose-glycine (II).‡ Such a reaction has not previously been observed though di-D-fructose-amino acids

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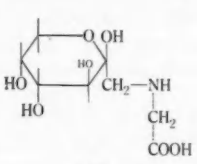
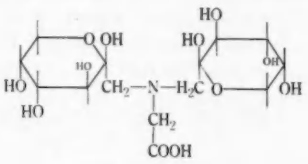
† The fully systematic name is *N*-(carboxymethyl)-1-amino-1-deoxy-D-fructose, the name 1-deoxy-1-glycino-D-fructose is also permissible although the prefix " glycino " is not specifically approved (Report on Nomenclature 1952).

‡ Two possible systematic names are *NN*-di-(D-*arabino*-3,4,5,6-tetrahydroxy-2-oxohexyl)-glycine and *NN*-di-(D-arabonoylmethyl)glycine, both these names imply the presence of a keto group which is not present as such in the compound.

(compounds F*a*) were detected but not recognized as such by Anet and Reynolds (1957) among the by-products of the reaction of D-glucose with amino acids. Also the compounds F1*a* and F4*a* found by them in extracts from browned fruit were probably di-D-fructose-L-aspartic acid and di-D-fructose-L-asparagine.




(I)




(II)

The formation of di-D-fructose-glycine was conveniently followed by paper electrophoresis in acid buffer. Non-ionic compounds, di-D-fructose-glycine, D-fructose-glycine, and glycine were well separated from each other. Displacement chromatography on a cation-exchange resin also gave a good separation and was used on a preparative scale. The rate of formation was found to increase with increasing pH and the decomposition of di-D-fructose-glycine was slower in slightly alkaline solution. The compound was prepared according to methods similar to those used (Anet 1957) for D-fructose-glycine but the best yield was obtained when the pH of the reaction mixture was increased by using the sodium salt of glycine instead of free glycine. D-Fructose-glycine could be substituted for glycine, and D-mannose for D-glucose. The product, a dihydrate $C_{14}H_{25}NO_{12}.2H_2O$, m.p. 112 °C, was obtained as colourless plates sparingly soluble in cold water.

TABLE 1

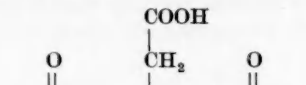
PERIODATE OXIDATIONS

Moles of periodate consumed

| Compounds | ½ Hour | 3 Hours | 7 Hours | 24 Hours |
|---|---|---|---|---|
| D-Fructose-glycine .. .. | 4·08 | 4·60 | 4·76 | 4·82 |
| Di-D-fructose-glycine .. | 6·06 | 7·62 | 7·86 | 7·86 |
| Di(carboxymethyl)amine .. | 0·57 | 0·99 | 1·09 | 1·26 |

Sodium metaperiodate reacted rapidly with di-D-fructose-glycine (Table 1). Eight molecules of reagent were consumed, two molecules of formaldehyde were formed, and tri(carboxymethyl)amine was isolated at the end of the reaction. D-Fructose-glycine on the other hand consumed five molecules of periodate and released two molecules of formaldehyde (cf. Richards 1956); further oxidation to glycine and formaldehyde of the di(carboxymethyl)amine formed would explain the consumption of one more molecule of oxidant and the production of the extra formaldehyde. It was found that di(carboxymethyl)amine reacted with one molecule of periodate and released one molecule of formaldehyde, but neither glycine nor tri(carboxymethyl)amine was oxidized under the same

conditions. The formation of tri(carboxymethyl)amine showed that in di-D-fructose-glycine the two sugar residues were attached to the nitrogen atom of



(III)

glycine by a terminal carbon atom adjacent to a keto or potential keto group as in structure III. Di-D-fructose-glycine is therefore the double Amadori rearrangement product and not a glycosylamine. This was supported by its formation from either D-glucose or D-mannose, showing that the asymmetry at carbon atom 2 of both sugar residues was lost. Also no glucose could be detected from the hydrolysis.

The ring form of the ketose in Amadori products is not definitely known (Hodge 1955). But 4,6-*O*-benzylidene-fructose-amine derivatives which cannot form a pyranose ring occur in the keto form as shown by their infra-red absorption in the region 1715–1725 $cm^{-1}$ (Micheel and Frowein 1957). It has now been found that the compound formed from D-xylose and glycine (Anet 1957), which

TABLE 2

INFRA-RED SPECTRA

Assignment of bands in the 2000–1500 $cm^{-1}$ region*

| Compound | Carbonyl | Carboxylate Ion | Unassigned |
|---|---|---|---|
| *N*-(carboxymethyl)-1-amino-1-deoxy-*keto*-D-*threo*pentulose .. .. | 1728 | 1618 | 1560 |
| D-Fructose-glycine .. .. .. | None | 1620 | None |
| Di-D-fructose-glycine .. .. .. | None | 1635 | None |
| Sodium salt of D-fructose-glycine† .. | None | 1626 | 1549 |
| Sarcosine‡ .. .. .. .. | None | 1616 | 1645 |

* All the absorption bands are shown in the table. They were all very strong.

† Spectra from Richards (1956).

‡ Spectra and assignments from Randall *et al.* (1949).

also cannot form a pyranose ring, showed a strong absorption at 1728 $cm^{-1}$ and therefore must be *N*-(carboxymethyl)-1-amino-1-deoxy-D-*threoketo*pentulose. In the above cases furanose forms could exist but must be less stable than the keto forms. Di-D-fructose-glycine and D-fructose-glycine show no peaks in the carbonyl region (Table 2) and must occur in a ring form, probably pyranose.

Richards (1956) proposed, from infra-red evidence, that the sodium salt of D-fructose-glycine had the 1,2-enol form. However, his results can be interpreted in a different way. The bands at 1618–1635 $cm^{-1}$ in ketose-amino acids (Table 2) are very strong and are unlikely to be due to the symmetrical C=C stretching. The same band is also present in the xylose derivative which is in the keto form

and is most probably due to the carboxylate ion. This absorption of the carboxylate ion in amino acids, for example sarcosine, is often outside the usual range 1550–1610 $cm^{-1}$ (Randall *et al.* 1949).

The molecular optical rotations per fructose unit at equilibrium of di-D-fructose-glycine and D-fructose-glycine were very similar, being 17,000 and 16,100 respectively. Thus their solutions probably contain much the same proportion of possible forms. Mutarotation could only be observed in the case of D-fructose-glycine, equilibrium being rapidly achieved (in 6–8 min) to give $[\alpha]_D^{25}$ $-67°$. The initial rotation was $[\alpha]_D^{25}$ $-73{\cdot}8°$ at $2\frac{1}{2}$ min. Derivatives of β-D-fructopyranose have large negative optical rotations, and those from both α-D-fructopyranose and *keto*-D-fructose have positive values (Barry and Honeyman 1952). Since the furanose forms seem to be the least stable in this series of compounds, the crystalline D-fructose-glycine probably occurs in the β-D-fructopyranose form (I). Di-D-fructose-glycine dihydrate probably is also a β-D-fructopyranose (II). All known crystalline D-fructose-amines probably have a β-D-configuration (Hodge 1955).

TABLE 3

DECOMPOSITION OF DI-D-FRUCTOSE-GLYCINE

Times for 2/3 decomposition

| Temperature (°C) | pH 3·5 | pH 5·5 | pH 8 |
|---|---|---|---|
| 25 | 50 days* | 9 days | 45 days*† |
| 40 | 70 hr | 25 hr | >100 hr‡ |
| 50 | 27 hr | 10 hr | 60 hr‡ |
| 75 | 45 min | 35 min | 130 min‡ |
| 100 | 4 min | 3 min | 35 min*‡ |

* Some loss of D-fructose-glycine was taking place.

† No neutral or acid compounds could be detected.

‡ An acid band was also present.

Although similar in most respects, di-D-fructose-glycine differs from D-fructose-glycine in being very much more labile. It decomposed spontaneously in solution to give a quantitative yield of D-fructose-glycine, the other fructose moiety being removed as unknown carbonyl compounds. No glucose, fructose, or hydroxymethylfurfuraldehyde could be detected. In slightly alkaline solution an acidic component was also formed. The rate of decomposition was studied at various pH values and temperatures (Table 3). The rate was maximal at pH 5·5 and at that pH the plot of the log of the time for 2/3 decomposition against the inverse of the absolute temperature gave a straight line. From this the activation energy was calculated to be 24,000 cal/mole.

Other diketose-amines were prepared in a similar way from other aldoses and other primary aliphatic amines, but the products could not be crystallized. These compounds decomposed in a similar way and at comparable rates to that of di-D-fructose-glycine, except that the compound derived from D-xylose was much less stable. This greater instability is probably due to the existence of

this compound in the keto form as in the case of the corresponding monoketose-amine (see above).

Several mechanisms have been suggested for the conversion of ketose-amino acids to brown polymers (Hodge 1953, 1955; Hurd and Buess 1956). These mechanisms depend on dehydration or aldol condensation and may be important under certain conditions or in the case of secondary amine derivatives. But in a system near neutrality ketose-amines are relatively stable compounds, being more stable than their glycosylamine precursors (Hodge 1955), and there is no evidence to show that their carbonyl groups are more reactive than those of the parent sugar. In the case of primary aliphatic amines (e.g. amino acids) a mechanism involving diketose-amines may well be more important, particularly when an excess of aldose is present. The reactions leading to diketose-amines which in turn quickly decompose back to a ketose-amine and carbonyl compounds provide a mechanism for the conversion of large amounts of aldoses to more reactive compounds, which would explain the partially catalytic role of amines in non-enzymic browning reactions.

A summary of some of the above work has already appeared (Anet 1958).

## II. Experimental

Melting points are uncorrected. Solutions were evaporated under reduced pressure using rotary evaporators. The microanalyses were carried out in the C.S.I.R.O. Microanalytical Laboratory at the University of Melbourne and the infra-red spectra by Dr. Durie, Coal Research Section, C.S.I.R.O.

(*a*) *Chromatography and Electrophoresis.*—Paper and ion-exchange chromatography were carried out as described in Part II of this series (Anet 1957). High voltage paper electrophoresis was used with Whatman 3MM paper strips, and acetic acid (5%) as buffer, for periods of 1 to 1½ hr at a potential gradient of 50 V/cm. At the end of a run the strips were dried at 50 °C for 20 min and then in front of a fan for 30 min at room temperature. The bands were revealed by using the same reagents as those used in the paper chromatography or the strips were dipped in the silver nitrate-sodium hydroxide reagent of Martin (1957) for quantitative determinations.

(*b*) *Preparation of Di-D-fructose-glycine.*—(i) *From D-Glucose and the Sodium Salt of Glycine.* Glycine (7·5 g), sodium hydroxide (4 g), and D-glucose (72 g) were dissolved in water and evaporated to a syrup containing approx. 20% water. The syrup was heated at 100 °C for 12 min when the pH after dilution with water was 8·0. The products were dissolved in ethanol (50%; 1 l.) and were separated by ion-exchange chromatography using 0·2M aqueous pyridine as displacing agent. Fractions 1–14, 18–21, and 23–24 contained essentially one compound each, *di-D-fructose-glycine*, D-fructose-glycine, and unchanged glycine respectively. Addition of acetone to fractions 1–14 yielded a brownish crystalline precipitate (8·5 g), 20% yield from the glycine. The crystals were quickly dissolved in a small volume of warm water, cooled, filtered, and allowed to stand at 1 °C. Di-D-fructose-glycine dihydrate crystallized as colourless plates (6·0 g), m.p. 111·5–112 °C, $[\alpha]_D^{25}$ —77° (no mutarotation after 3 min, *c*, 0·5 in water) (Found: C, 38·2; H, 6·9; N, 3·0; O, 51·3%. Calc. for $C_{14}H_{25}NO_{12}.2H_2O$: C, 38·6; H, 6·7; N, 3·2; O, 51·5%). Drying gave the anhydrous compound, m.p. 144–146 °C (decomp.) (Found: C, 41·7; H, 6·4; N, 3·5; O, 48·4%. Calc. for $C_{14}H_{25}NO_{12}$: C, 42·1; H, 6·3; N, 3·5; O, 48·1%).

(ii) *From D-Mannose and the Sodium Salt of D-Fructose-glycine.* D-Fructose-glycine (3 g), D-mannose (6 g), and sodium hydroxide (0·5 g) were dissolved in water and evaporated to a syrup. The syrup was heated at 100 °C for 6½ min. The products were separated as in (i) above. The yield of di-D-fructose-glycine dihydrate after one recrystallization from aqueous ethanol was 2 g (40% of D-fructose-glycine), colourless plates, m.p. 112–113 °C, $[\alpha]_D^{25}$ —78° (*c*, 0·5 in water) (Found after drying: C, 41·6; H, 6·6; N, 3·4; O, 48·6%. Calc. for $C_{14}H_{25}NO_{12}$: C, 42·1; H, 6·3; N, 3·5; O, 48·1%).

(iii) *From* D-*Glucose and Glycine in the Presence of Bisulphite.* The reaction was carried out with mechanical stirring in a 2 l. beaker partially immersed in a boiling water-bath. D-Glucose (720 g) was gradually added to 100 ml of hot water. When the temperature reached 95 °C sodium metabisulphite (95 g) was added followed by glycine (75 g). The mixture was heated for 1 hr then water (2 l.) was added and the solution was diluted to 4·5 l. with degassed ethanol (95%). Four batches were combined and the product separated by ion-exchange chromatography. The fractions emerging before glycine were combined and yielded pure D-fructose-glycine (over 200 g, 20% conversion of glycine) after fractional crystallization from aqueous ethanol. The mother liquors yielded a syrup (50–60 g) which was rechromatographed. From the first few fractions pure di-D-fructose-glycine dihydrate (2 g) was obtained by crystallization from aqueous ethanol, m.p. 111·5–112 °C, $[\alpha]_D^{25}$ —78° (*c*, 1 in water) (Found after drying: C, 41·6; H, 6·4; N, 3·4; O, 48·4%. Calc. for $C_{14}H_{25}NO_{12}$: C, 42·1; H, 6·3; N, 3·5; O, 48·1%).

(*c*) *Some Properties of Di-*D-*fructose-glycine.*—(i) *Ion-Exchange Columns.* Displacement chromatography on sulphonated polystyrene resin (4% cross-linkage) gave a sharp separation of di-D-fructose-glycine from D-fructose-glycine and from glycine. The compounds emerged in that order.

(ii) *Paper Chromatography.* Owing to its low $R_F$ value in the solvents used, several days were required for di-D-fructose-glycine to move from the starting line, during which time some decomposition took place. The $R_F$ value in butanol–acetic acid–water (4 : 1 : 1 v/v) was 0·48 that of D-fructose-glycine. The colour reactions on paper with ninhydrin, triphenyltetrazolium chloride–sodium hydroxide, alkaline silver nitrate, and aniline phosphate were similar to those described for D-fructose-glycine (Anet 1957).

(iii) *Paper Electrophoresis.* Di-D-fructose-glycine gave only one band which had a mobility of approx. half that of D-fructose-glycine in acetic acid (5%) buffer.

(iv) *Solubility.* Pure di-D-fructose-glycine was only sparingly soluble in cold water but dissolved readily in warm water, from which it crystallized as the dihydrate on cooling or on addition of ethanol or acetone. It was insoluble in anhydrous organic solvents.

(v) *Basic Strength.* Di-D-fructose-glycine was found to have $pK_b$ 5·4 compared to those of D-fructose-glycine 8·4 and glycine 9·6. A 0·1M solution had pH 3·63 compared to D-fructose-glycine 5·47 and glycine 6·0 under the same conditions.

(vi) Infra-red spectra were recorded using potassium chloride disks and sodium chloride optics on a Perkin-Elmer model 21 infra-red spectrophotometer.

(*d*) *Periodate Oxidation.*—(i) *Determination of Periodate Consumption.* An equal volume of sodium metaperiodate (0·05M) was added to a solution of each of the following: D-fructose-glycine (0·005M), di-D-fructose-glycine (0·0025M), glycine (0·025M), di(carboxymethyl)amine (0·025M), and tri(carboxymethyl)amine (0·025M). The mixtures were allowed to stand at room temperature in the dark. At intervals the consumption of periodate was determined with iodine (0·01N) after the addition of excess neutral arsenite (Jackson 1944). Glycine and tri(carboxymethyl)amine consumed very little periodate. The results for the other compounds are shown in Table 1.

(ii) *Formation of Formaldehyde.* The production of formaldehyde was determined as the dimedone derivative, according to the method of Reeves (1941) except that 4 hr was allowed for the oxidation. The formaldehyde dimedone derivatives all had the same m.p. 190 °C undepressed by an authentic specimen. Means of triplicate determinations gave 1·88, 2·12, and 0·97 moles of formaldehyde per mole of D-fructose-glycine, di-D-fructose-glycine, and di(carboxymethyl)-amine respectively.

(iii) *Isolation of Tri(carboxymethyl)amine.* Di-D-fructose-glycine (1 g) and sodium metaperiodate (4·28 g) in water (200 ml) were allowed to react in the dark at room temperature for 3 hr. The solution was then passed through a column of sulphonated polystyrene resin and the effluent was treated with barium hydroxide to precipitate the iodic acid. The filtrate was concentrated, filtered, and allowed to crystallize after the addition of ethanol, yielding prisms, m.p. 240–242 °C (decomp.), undepressed with an authentic specimen (Found: N, 7·2%. Calc. for $C_6H_9NO_6$: N, 7·3%).

(e) *Spontaneous Decomposition of Di-D-fructose-glycine in Solution.*—(i) *Isolation of D-Fructose-glycine.* Di-D-fructose-glycine (250 mg) in water (25 ml) was kept at 50 °C for 4 days, then diluted with an equal volume of ethanol (95%). The solution was passed through a column of sulphonated polystyrene resin (0·4 g), which was washed with ethanol (50%; 50 ml) and the products were displaced with 0·2M aqueous pyridine. The effluent was evaporated to dryness, dissolved in a small volume of water, and ethanol added to slight turbidity. D-Fructose-glycine crystallized overnight in approx. quantitative yield, m.p. 145 °C, $[\alpha]_D^{25}$ —73·8° (2½ min) —67° (6–8 min constant, *c*, 2·25 in water) (cf. Anet 1957; Dubourg and Devillers 1957) (Found: C, 40·4; H, 6·3; N, 5·6%. Calc. for $C_8H_{15}NO_7$: C, 40·5; H, 6·3; N, 5·9%).

(ii) *Non-Nitrogenous Products.* An aqueous solution of di-D-fructose-glycine (0·005M) adjusted to pH 4·6 with pyridine was heated at 100 °C for 3 min, the pH rose to 5·0. The solution was deionized with sulphonated polystyrene resin. The resulting solution showed a sharp maximum at 233 mμ ($\varepsilon$=1300 assuming mole for mole formation). Heating the deionized solution at 100 °C only gave a broadening of the absorption peak, however, in weakly acid media (0·03N acetic acid) after 1 hr at 100 °C a new peak was formed (max. 284 mμ, $\varepsilon$=1300 assuming mole for mole formation from the di-D-fructose-glycine) and the 233 mμ peak disappeared.

At lower temperatures the maximum was lower and not so sharp. In weakly alkaline solution a very broad peak was observed, maximum 295 mμ, but no 233 mμ maximum.

(iii) *Rate of Decomposition.* Di-D-fructose-glycine (15 mg) in a phosphate-citrate buffer (8 ml) was allowed to stand in the dark at various temperatures. At intervals, samples were withdrawn and subjected to quantitative paper electrophoresis. Three bands were seen, one due to neutral compounds, and two due to cations of different mobility, corresponding to di-D-fructose-glycine and D-fructose-glycine. When the two mobile bands were of equal intensity 2/3 decomposition had taken place. This point could easily be observed. Solutions containing di-D-fructose-glycine and D-fructose-glycine in the ratio 1 : 2 molar showed two bands of equal intensity with the silver nitrate reagent, when subjected to paper electrophoresis. At 50 °C the maximum rate of decomposition was found to be at pH 5·5. The times for 2/3 decomposition at pH 3·5, 5·5, and 8 at various temperatures are shown in Table 3.

(f) *Formation and Stability of Diketose-amines.*—(i) Sugar (1–4 mmoles) and amine (1 mmole) were dissolved in a solution of sodium hydroxide (0–1 mmole) and concentrated to a syrup. The syrup was heated at 100 °C for 3–20 min, diluted with water (3 ml), and 1 to 2 streaks applied to a strip of paper and subjected to paper electrophoresis. The best yields were obtained when the pH of the reaction mixture did not fall below 8.

Three separate reducing bands were detected on electrophoresis from the reaction of glycine with any of the aldose sugars, D-glucose, D-mannose, D-galactose, and D-xylose. These bands corresponded to the neutral compounds, the diketose-amine and the ketose-amine. D-Xylose reacted much more quickly than the aldohexoses. D-Glucose reacted with 1-deoxy-1-glycino-α-D-glucose (Anet 1957) to give D-glucose-D-fructose-glycine, however, D-fructose did not react with D-fructose-glycine, when the same compound could have been formed. When D-fructose-glycine was substituted for glycine the diketose-glycine band was presumably a mixture in the case of sugars other than D-glucose or D-mannose, since large amounts of the mono-D-tagatose-glycine were formed in the case of galactose (see following paragraph). Glucose reacted to give di-D-fructose-amines with other amino acids, the reaction with β-alanine was slightly faster than with glycine, but α-alanine, aspartic acid, and asparagine reacted much more slowly.

(ii) The reactions of D-glucose with β-alanine, D-glucose with ethylamine, D-xylose with glycine, D-galactose with glycine, and D-galactose with D-fructose-glycine were carried out on a larger scale as in Section II (*b*) (i) or (ii). The products from the reaction of D-xylose were separated at 1 °C, as the diketose compound decomposed rapidly at room temperature. The mono- and diketose-amines were easily separated but none of the products crystallized. Paper chromatography and paper electrophoresis confirmed the presence of both D-tagatose-glycine and D-fructose-glycine in the monoketose-amine fractions from the reaction of D-fructose-glycine and D-galactose. The tagatose compound had a higher $R_F$ value and ionic mobility than that of D-fructose-glycine and the colour given with the silver nitrate reagent was much blacker.

(iii) The stability of solutions of both di-D-tagatose-glycine and di-D-fructose-β-alanine from the above preparations was studied at pH 3·5, 5·5, and 8 at 75 °C as in Section II (*e*) (iii); the times for 2/3 decomposition were 26, 11½, 100 min, and 25, 19, and 90 min respectively.

## III. Acknowledgments

The author is indebted to Dr. T. M. Reynolds for helpful discussion and to Miss Marie Herron for technical assistance.

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# CHEMISTRY OF NON-ENZYMIC BROWNING

## VIII. THE HYDROLYTIC REACTIONS OF ALDOSE BISULPHITE ADDITION COMPOUNDS

By D. L. INGLES*

[*Manuscript received December* 1, 1958]

*Summary*

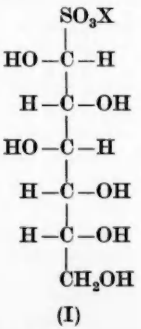
A study has been made of the hydrolytic reactions of aldose bisulphite addition compounds (Ingles 1959*a*, 1959*b*). Addition compounds which had a hydroxyl group at $C_1$ were stable in 10 per cent. acetic acid but decomposed in water and sodium hydroxide forming the α-form of the parent D-aldose. Compounds in which the hydroxyl at $C_1$ was replaced by an amine group were unstable in water, 10 per cent. acetic acid, and sodium hydroxide. In the last solvent breakdown to the α-form of the D-glycosylamine was observed.

Decomposition in sodium hydroxide appears to occur with inversion of configuration at $C_1$. The configurations at $C_1$ of the bisulphite addition compounds have been listed.

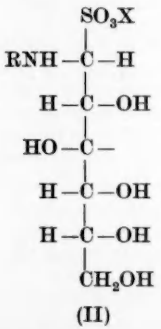
### I. INTRODUCTION

The preparation of a series of aldose potassium bisulphite addition compounds and some amine derivatives has been described (Ingles 1959*a*). Another paper (Ingles 1959*b*) described the compounds obtained from the reaction of aldoses with amine bisulphites. These compounds undergo complex hydrolytic reactions which have been studied polarimetrically. This study has provided information on the stability of the addition compounds in dilute aqueous solution and on the configuration at the $C_1$ position.

The compounds studied were of two types, I and II, formulated for the D-glucose reaction products. In the compounds of type I, X was sodium,



(I)



(II)

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potassium, or *cyclo*hexylammonium. In the compounds of type II, X was sodium, potassium, or *cyclo*hexylammonium while R was phenyl, *p*-tolyl, *p*-methoxyphenyl, and *o*-aminophenyl. The aldoses from which these compounds have been prepared include D-glucose, D-galactose, L-arabinose, D-mannose, and L-rhamnose.

The systematic name for the glucose derivative I (X=potassium) is D-*glycero*-D-*ido*-1,2,3,4,5,6-hexahydroxylhexyl potassium sulphonate (Rules of Carbohydrate Nomenclature 1952, rules 6 and 22). The trivial name glucose potassium sulphonate is also used. The compound II (X=potassium, R=phenyl)

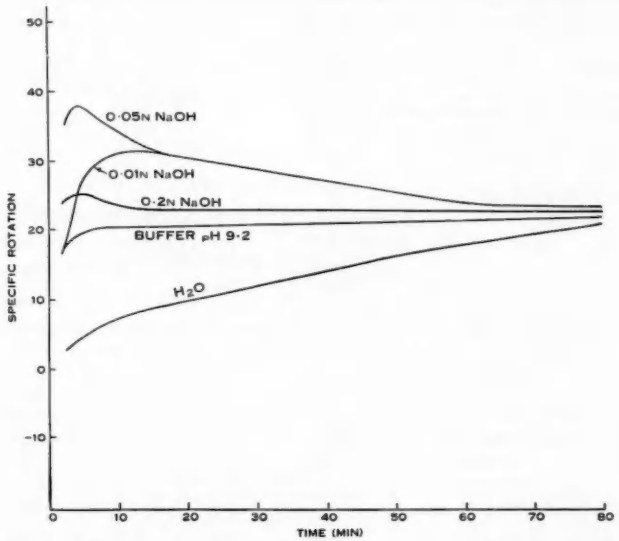


Fig. 1.—Specific rotation (25 °C) of glucose potassium sulphonate (c, 0·1M).

is named D-*glycero*-D-*ido*-1-anilino-2,3,4,5,6-pentahydroxylhexyl potassium sulphonate. Other compounds are named similarly. The systematic naming defines the configuration at the $C_1$ position and is based on the results given below.

The hydrolytic reactions of the addition compounds were studied in water, 10 per cent. acetic acid, and aqueous sodium hydroxide of varying normality. The changes of specific rotation with time were followed with solutions containing approximately 2–4 per cent. of addition compound.

Glucose potassium sulphonate I (X=potassium) decomposed in water (Fig. 1) to give an equilibrium at which hydrolysis was approximately 85 per cent. complete. The extent of hydrolysis depends upon pH and concentration (cf. Braverman 1953). The rate of hydrolysis of this compound was markedly increased by sodium hydroxide, the effect being maximal with 0·05N sodium

hydroxide (Fig. 1) which gave a peak specific rotation ($[\alpha]_D^{25}=37\cdot7°$) in 4 min. The curve then followed a negative slope until equilibrium was reached. The peak specific rotation indicates that the high rotating, or α, form of D-glucose was liberated by the sodium hydroxide. Mutarotation of the α-D-glucose then resulted in a negative slope until equilibrium of the α- and β-forms of D-glucose was attained. Increasing concentrations of sodium hydroxide gave lower peak specific rotations. The peak values observed depend upon the relative rates of hydrolysis of the glucose potassium sulphonate and of mutarotation of the liberated α-D-glucose. Only when the rate of hydrolysis is faster than the rate of mutarotation is a peak observed. Since the rate of mutarotation of α-D-glucose is rapid at high pH values (>7·0) no peak in the curve was obtained in buffer

TABLE 1

SPECIFIC ROTATION AT 25 °C OF TYPE I COMPOUNDS

| Type I Compound | | Solvent | | | |
|---|---|---|---|---|---|
| Parent Aldose | X= | 10% Acetic Acid Initial Value (extrapolated) | 0·05N Sodium Hydroxide | | Configuration at $C_1$ |
| | | | Peak Value Max. or Min. | Equilibrium Value | |
| D-Glucose .. | Sodium | —3·9 | 32·8 (4 min) | 29·5 (30 min) | β |
| | Potassium | —4·8 | 37·7 (4 min) | 26·6 ( 1 hr) | β |
| | *cyclo*Hexylamine | —7·0 | 23·5 (2 min) | 20·8 (15 min) | β |
| D-Galactose .. | Potassium | 7·7 | 50·0 (7 min) | 45·0 (30 min) | β |
| L-Arabinose .. | Potassium | 13·0 | 44·0 (3 min) | 41·8 (12 min) | α |
| D-Mannose .. | Potassium | 7·2 | 9·8 (2 min) | 7·3 (13 min) | β |
| L-Rhamnose .. | Potassium | 1·7 | 0·9 (4 min) | 3·8 (20 min) | β |

of pH 9·2. Hydrolysis with sodium hydroxide produces not only α-D-glucose but also potassium bisulphite which neutralizes the sodium hydroxide with a consequent lowering of pH and of the rate of mutarotation of the α-D-glucose. Increasing the initial concentration of sodium hydroxide from 0·05 to 0·2N led to an excess of sodium hydroxide after hydrolysis, which caused a faster mutarotation reaction and thus a lower peak specific rotation.

Similar results were found with the sulphonates (I) derived from other aldoses and from other cations (Table 1). In each case a peak specific rotation was observed in 0·05N sodium hydroxide. With the potassium sulphonate derived from L-rhamnose a minimum value ($[\alpha]_D^{25}=0\cdot9°$) was found at 4 min. This was followed by an increase in rotation until equilibrium was reached, indicating the formation of α-L-rhamnose which has a specific rotation $[\alpha]_D^{25}$ $-8\cdot6°\rightarrow8\cdot2°$. Arabinose potassium sulphonate hydrolysed to form the high rotating, or β, form of L-arabinose.

Whereas compounds of type I were readily hydrolysed in water and sodium hydroxide they were relatively stable in 10 per cent. acetic acid (see Fig. 2 and Table 1). The potassium sulphonates of mannose and rhamnose showed no change in rotation even after 24 hr. The compounds derived from arabinose, galactose, and glucose hydrolysed only very slowly. Glucose *cyclo*hexylammonium sulphonate behaved similarly in this solvent.

Compounds of type II, however, were unstable in 10 per cent. acetic acid, hydrolysing rapidly until equilibrium was attained (Fig. 3 and Table 2). The aniline and *p*-toluidine derivatives hydrolysed rapidly, the equilibrium rotation indicating almost complete conversion to glucose. The *p*-anisidine derivative

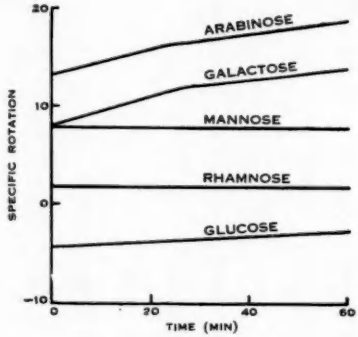


Fig. 2

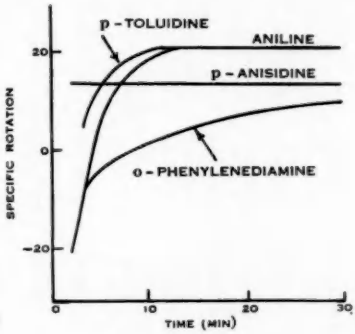


Fig. 3

Fig. 2.—Specific rotation (25 °C) of aldose potassium sulphonates (*c*, 3%) in 10% acetic acid.

Fig. 3.—Specific rotation (25 °C) of the amine derivatives of glucose potassium sulphonate (*c*, 3%) in 10% acetic acid.

hydrolysed immediately and the *o*-phenylenediamine derivative more slowly. In both cases the final rotation ($[\alpha]_D^{25}$ approx. 13°) indicated an equilibrium mixture. Other compounds of this type behaved similarly. Their initial rotations in water are shown in Table 2.

The rate of hydrolysis of type II compounds was greatly increased by sodium hydroxide. This hydrolysis differed from that in acetic acid in that it stopped at the *N*-substituted glycosylamine which was stable in alkaline solution. The specific rotation of the aniline derivative of glucose potassium sulphonate II (X = potassium, R = phenyl) in 0·05N sodium hydroxide reached a maximum ($[\alpha]_D^{25}$ 35·0°) in 5 min. The curve then followed a negative slope until equilibrium ($[\alpha]_D^{25}$ −58°) was attained. Increasing the concentration of sodium hydroxide from 0·05N to 1·0N slightly increased the rate of hydrolysis but had a greater effect on the rate of mutarotation (Fig. 4). The peak values were progressively lowered. The rate of mutarotation of synthetic *N*-phenyl-D-glucosylamine in sodium hydroxide showed a similar increase when the concentration was increased from 0·01 to 1·0N. These results indicate the formation of the high rotating, or α, form of *N*-phenyl-D-glucosylamine. Mutarotation of this compound to

TABLE 2

SPECIFIC ROTATION AT 25 °C OF TYPE II COMPOUNDS

| Type II Compound | | Solvent | | | | Configuration at $C_1$ |
|---|---|---|---|---|---|---|
| | | Water | 0·1N Sodium Hydroxide | | 10% Acetic Acid | |
| Parent Aldose | X= R= | Initial | Peak Value Max. or Min. | Equilibrium Value | | |
| D-Glucose .. | Potassium Phenyl | —69·3 (2 min) | 29·0 (3 min) | —58·0 (1 hr) | —14·3 (3 min)→22·0 (30 min) | β |
| D-Glucose .. | Sodium Phenyl | —22·6 (1 min) | —29·5 (7 min) | —56·2 (1 hr) | —6·3 (4 min)→23·0 (30 min) | β |
| D-Glucose .. | Potassium *p*-Tolyl | —45·9 (1 min) | 9·0 (3 min) | —41·0 (1½ hr) | 4·0 (3 min) →22·0 (30 min) | β |
| D-Glucose .. | Sodium *p*-Tolyl | —40·8 (1 min) | —34·0 (10 min) | —41·2 (1 hr) | —1·1 (4 min)→22·0 (30 min) | β |
| D-Glucose .. | Potassium *p*-Methoxyphenyl | —36·4 (1 min) | 12·4 (2 min) | —41·5 (1 hr) | 13·0 constant | β |
| D-Glucose .. | Potassium *o*-Aminophenyl | —13·0 (2 min) | —11·6 (4 min) | —17·5 (½ hr) | —5·2 (4 min)→12·5 (40 min) | β |
| D-Glucose .. | Anilinium Phenyl | —51·2 (10 min) | 2·9 (10 min) | —46·5 (19 hr) | —7·5 (5 min)→17·6 (30 min) | β |
| D-Glucose .. | Piperidinium Phenyl | —69·5 (1 min) | 19·3 (3 min) | —50·0 (2 hr) | —37·5 (1 min)→21·6 (15 min) | β |
| D-Glucose .. | *cyclo*Hexylammonium Phenyl | —69·0 (2 min) | 20·8 (3 min) | —50·2 (2 hr) | —15·0 (3 min)→18·7 (2 hr) | β |
| D-Galactose .. | Anilinium Phenyl | —40·7 (2 min) | 3·3 (19 min) | —26·7 (2 hr) | 11·0 (2 min)→28·6 (15 min) | β |
| D-Mannose .. | Anilinium Phenyl | 52·9 (7 min) | — | —46·2 (16 hr) | 32·0 (7 min)→14·3 (2 hr) | — |

give an equilibrium of α- and β-forms results in the negative slope in the specific rotation curve.

The equilibrium specific rotation finally attained ($[\alpha]_D^{25}$ −58·0°) compares reasonably with the calculated specific rotation $[\alpha]_D^{25}$ −55·5° (cf. Pigman *et al.* 1951) assuming complete conversion to *N*-phenyl-D-glucosylamine. Similarly the equilibrium specific rotation obtained for the *p*-toluidine derivative of glucose potassium sulphonate in 0·1N sodium hydroxide ($[\alpha]_D^{25} = -41·0°$) agrees with the calculated rotation ($[\alpha]_D^{25}$ −41·6°) assuming complete conversion to *N*-*p*-tolyl-D-glucosylamine. The results obtained with other compounds of type II are shown in Table 2. In all but one instance a peak rotation was observed. The

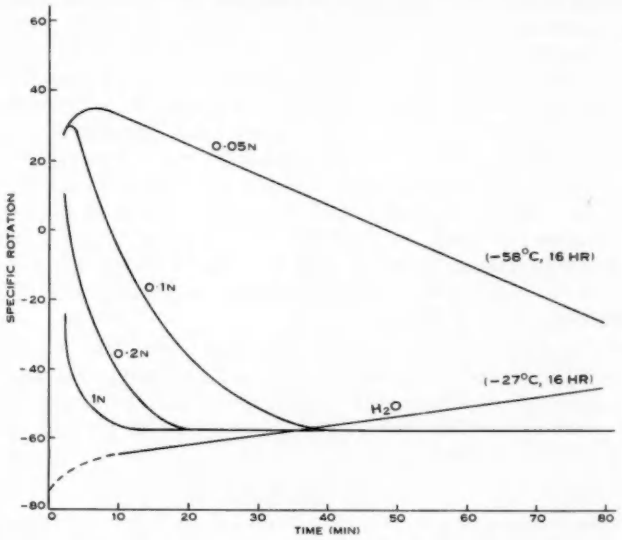


Fig. 4.—Specific rotation (25 °C) of the aniline derivative of glucose potassium sulphonate (*c*, 2%) in sodium hydroxide.

exception was the compound II (X=anilinium, R=phenyl) derived from D-mannose by reaction with aniline bisulphite. This compound had a high positive rotation and hydrolysed slowly with sodium hydroxide but without showing a peak in the hydrolysis curve. Increasing the sodium hydroxide concentration gave a faster hydrolysis but any *N*-phenyl-D-mannosylamine liberated apparently mutarotated even more rapidly.

The high positive rotation ($[\alpha]_D^{25}$ 52·9°) (Table 2) of the mannose derivative II (X=anilinium, R=phenyl) suggests that this compound has the α-configuration at $C_1$. Similarly the low negative rotation of the glucose derivative II (X=anilinium, R=phenyl) suggests the β-configuration at $C_1$. This designation of the $C_1$ configuration as α or β is based on the usage proposed by Hudson (1909)

in which the compound of a D series having the more positive rotation is assigned the prefix α (cf. also Dimler and Link 1940; Wolfrom, Konigsberg, and Moody 1940). It is noteworthy that in all the solvents used the mannose compound showed a decreasing rotation and the glucose derivative an increasing rotation, changes also associated with α- and β-isomers respectively. The formulation II (X=anilinium, R=phenyl) for the D-glucose derivative thus shows the aniline moiety to the left as for a β-isomer. The $C_1$ configurations suggested for the other compounds of type II are listed in Table 2.

The formation of *N*-phenyl-α-D-glucosylamine by the action of sodium hydroxide on the glucose derivative II (X=anilinium, R=phenyl) indicates that a Walden inversion has occurred at the $C_1$ position. The role of neighbouring groups in such replacement reactions has been discussed by Winstein and Buckles (1942) and more recently by Isbell and Frush (1949) in their theory of the formation of diacetamido L-arabinose. Hibbert and Carter (1929) considered that in such replacement reactions a neighbouring nucleophilic group approaches the face of a carbon atom opposite a replaceable group, combining with inversion of configuration and with the release of electrons to the replaceable group. It is suggested that in the presence of sodium hydroxide, the $C_5$ hydroxyl group of the glucose derivative II (X=anilinium, R=phenyl) approaches the $C_1$ position, replacing the sulphonate group, and forming *N*-phenyl-α-D-glucosylamine. Similar considerations apply to the other compounds of type II examined in sodium hydroxide solution (Table 2), with the possible exception of the mannose derivative II (X=anilinium, R=phenyl) where the formation of *N*-phenyl-β-D-mannosylamine was not observed.

It has been shown above that glucose potassium sulphonate I (X=potassium) forms α-D-glucose in sodium hydroxide solution. Assuming that here also the action of alkali causes inversion of the configuration at $C_1$, the addition compound will have the β-form. The appropriate $C_1$ configurations for such compounds (type I) are shown in Table 1.

## II. Experimental

Specific rotations were followed in a Hilger Polarimeter at 25 °C using 2 dm tubes. The aldose bisulphite addition compounds studied were prepared as previously described (Ingles 1959*a*, 1959*b*).

Compounds of type I were studied as approx. 3% solutions in water, sodium hydroxide (0·01, 0·05, 0·2N), sodium borate buffer (pH 9·2), and 10% acetic acid (Figs. 1, 2, and Table 1). Compounds of type II were studied as approx. 2% solutions in water, sodium hydroxide (0·05, 0·1, 0·2, 1·0N) and 10% acetic acid (Figs. 3, 4, and Table 2).

The equilibrium rotation of *N*-*p*-tolyl-glucosylamine (prepared according to Sorokin 1887) in 0·1N sodium hydroxide was determined, $[\alpha]_D^{25}$ −63° (*c*, 2).

The specific rotations of *N*-phenyl-D-glucosylamine in 0·01N and 1·0N sodium hydroxide were respectively $[\alpha]_D^{25}$ −3·6° (7 min) →−83·2° (const. 16 hr, *c*, 2·2), $[\alpha]_D^{25}$ −48° (5 min) →−83·2° (const. 15 min, *c*, 1·8). Pigman *et al.* (1951) finds $[\alpha]_D^{25}$ 37·2° (initial) →−85·6° (final) for *N*-phenyl-D-glucosylamine in 0·01N sodium hydroxide.

## III. Acknowledgment

The author is indebted to Dr. T. M. Reynolds for helpful discussion.

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# THE PREPARATION OF COMPOUNDS RELATED TO *S*–2–AMINOETHYL–L–CYSTEINE

By H. LINDLEY*

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*Summary*

The present paper describes the preparation of benzyloxycarbonyl-2-bromoethylamine, benzyloxycarbonyl - 2 - iodoethylamine, *S* - 2 - benzyloxycarbonylaminoethyl - L-cysteine, *S*-2-aminoethyl-L-cysteine, and polymers of the last two compounds. The preparation of carboxymethyl 2-aminoethyl sulphide is also described and it is reported that the latter compound does not act as an inhibitor for the hydrolysis of toluene-*p*-sulphonyl-L-arginine methyl ester by trypsin.

## I. INTRODUCTION

The motive underlying the present work was to determine whether the structural similarity of the side chains of lysine and *S*-2-aminoethyl cysteine was sufficiently close for the enzyme trypsin to hydrolyse peptide bonds involving the latter amino acid. A preliminary account showing that the polymer of *S*-2-aminoethyl cysteine is an excellent substrate for trypsin has already been published (Lindley 1956), and the present paper gives details of the compounds prepared in connection with the study. *S*-2-Aminoethyl cysteine had already been prepared at the time this work was undertaken (Cavillini *et al.* 1955) by direct coupling of cysteine and 2-bromoethylamine. However, for the preparation of the polymer it was more desirable to use *S*-(benzyloxycarbonylaminoethyl) cysteine as the key compound and hence the present synthesis was via this intermediate.

It was proposed to make use of the trypsin susceptibility of peptide bonds involving *S*-aminoethyl cysteine residues by first reducing the cystine residues of proteins with mercaptoacetate ("thioglycollate") followed by coupling the thiol groups of the reduced protein with bromoethylamine. It was therefore necessary to study the reaction between mercaptoacetic acid and bromoethylamine to see whether the product of this reaction, carboxymethyl 2-aminoethyl sulphide, could act as a trypsin inhibitor.

## II. EXPERIMENTAL

All melting points are uncorrected and analyses are by the C.S.I.R.O. Microanalytical Laboratory.

(*a*) *Preparation of Benzyloxycarbonyl-2-bromoethylamine.*†—2-Bromoethylamine hydrobromide (10 g) and 4·2 g sodium bicarbonate were dissolved in 100 ml water and the solution cooled in an ice-bath. Benzyloxycarbonyl chloride (9 ml) was added followed by N sodium hydroxide (50 ml) in 10 ml portions with vigorous shaking after each addition. The product solidified after the

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† *Note added in Proof.*—It has now been realized that benzyloxycarbonyl-2-bromoethylamine and also the iodo derivative have been previously prepared (Katchalski and Ben Ishai 1950). These authors record melting points of 45 and 69° C respectively.

final portion of alkali had been added. (This procedure was followed rather than the conventional one of simultaneous addition of approximately equivalent amounts of the acid chloride and alkali because of the ease with which 2-bromoethylamine is converted to ethyleneimine hydrobromide under alkaline conditions. If the bromoethylamine was allowed to remain in alkaline solution before adding the benzyloxycarbonyl chloride, the sole product was a lachrymatory oil which was not further investigated but is presumably the *N*-benzyloxycarbonyl derivative of ethyleneimine.) After separation and washing by decantation the *product* was dissolved in ethyl acetate and the solution dried over calcium chloride. The product was recovered from solution by evaporation of the solvent in a vacuum and recrystallized as long needles from light petroleum (b.p. 40–60 °C), m.p. 47–49 °C (Found : C, 47·2 ; H, 4·8 ; N, 5·4 ; Br, 31·2%. Calc. for $C_{10}H_{12}O_2NBr$ : C, 46·5 ; H, 4·7 ; N, 5·4 ; Br, 31·0%).

(*b*) *Benzyloxycarbonyl-2-iodoethylamine.*—One equiv. of benzyloxycarbonyl-2-bromoethylamine was refluxed for 1 hr in acetone with 1 equiv. of sodium iodide. After cooling the solution was filtered to remove sodium bromide and the acetone removed by vacuum distillation. The *product* crystallizes as long needles from light petroleum (b.p. 40–60 °C), m.p. 66–67 °C (Found : C, 39·6 ; H, 4·1 ; N, 4·2 ; I, 40·5%. Calc. for $C_{10}H_{12}O_2NI$ : C, 39·3 ; H, 4·0 ; N, 4·6 ; I, 41·6%).

(*c*) S-*Benzyloxycarbonylaminoethyl-*L-*cysteine.*—(i) *By Coupling in Liquid Ammonia.* L-Cystine (4·6 g) was suspended in liquid ammonia (150 ml) and reduced to cysteine by metallic sodium. As soon as a permanent blue colour was obtained indicating excess sodium, this was discharged by the addition of the minimal quantity of ammonium chloride. The solution was then cooled in a solid $CO_2$–ethanol bath and benzyloxycarbonyl-2-bromoethylamine (10 g) added in small portions. After the final addition the ammonia was allowed to evaporate overnight. The solid product was dissolved in water and the solution filtered and the pH adjusted to 4 with hydrochloric acid. The product precipitated and was filtered and washed successively with water, ethanol, and ether. Recrystallization was carried out by dissolving the material in the minimal volume of hot N hydrochloric acid, diluting with 5 vol boiling water, and then adding a hot solution of sodium acetate (2 equiv. based on HCl used). The *product* crystallized as lustrous plates on cooling, m.p. 204–205 °C (decomp.). Yield (recrystallized product) 10·1 g (Found : C, 52·3 ; H, 6·1 ; O, 22·3 ; N, 8·9 ; S, 10·7%. Calc. for $C_{13}H_{18}O_4N_2S$ : C, 52·3 ; H, 6·1 ; O, 21·4 ; N, 9·4 ; S, 10·7%).

(ii) *By Coupling in Aqueous Alcohol.* L-Cysteine hydrochloride (1·6 g) was dissolved in 50 ml water and a solution of benzyloxycarbonyl-2-iodoethylamine (3·25 g) in ethanol (100 ml) was added. A few drops of phenolphthalein were added and the solution titrated to a pink colour with 5N sodium hydroxide and left stirring for 30 min. The solution was then acidified to pH 4 and the precipitated product filtered and recrystallized as in Section II (*c*) (i). Yield 1·8 g.

The product could also be prepared using benzyloxycarbonyl-2-bromoethylamine instead of the iodo compound. Reaction in this case was slower and required 4 hr at 30 °C.

(*d*) S-*2-Aminoethyl-*L-*cysteine Hydrochloride.*—Treatment of the preceding compound with anhydrous hydrobromic acid (6N) in acetic acid caused quantitative removal of the benzyloxycarbonyl group in 30 min. Precipitation of the product was assisted by the addition of ether prior to filtration. The hygroscopic product was dissolved in water and converted to the free amino acid by passage through a column of " Amberlite 1R4 " in the basic form. The alkaline eluate (pH 9) was titrated to pH 4 with hydrochloric acid and evaporated to dryness in a vacuum. The *S*-2-aminoethyl cysteine hydrochloride was crystallized by dissolving in twice its weight of water and adding an equal volume of hot ethanol to the hot solution (Found : C, 29·8 ; H, 6·5 ; N, 13·5 ; S, 15·6 ; Cl, 17·8%. Calc. for $C_5H_{13}O_2N_2SCl$ : C, 29·9 ; H, 6·5 ; N, 14·0 ; S, 16·0 ; Cl, 17·7%) ; m.p. 195 °C (decomp.), $[\alpha]_D^{16}$ —4·4° (*c*, 3% in $H_2O$). Cavallini *et al.* give m.p. 192–192·5 °C, $[\alpha]_D^{25}$ +7·2° (*c*, 1% in $H_2O$). No reason can be advanced for the discrepancy in optical rotation found in the present work and that reported by Cavillini *et al.* (1955). Dr. S. J. Leach kindly determined the cystine content of the sample of *S*-aminoethyl cysteine used for optical rotation measurements by amperometric titration and showed that it was less than 0·1%. Therefore, the discrepancy cannot be explained on the basis of contamination of the present sample with cystine.

A titration curve was carried out on the sample and gave 1·93 for the pK of the carboxyl group (25°). Calculated pK values for the amino groups were 8·8 and 9·2 but the curve showed little trace of any inflection.

(*e*) *Polymer of* S-*2-Aminoethyl-*L*-cysteine.*—The initial procedure outlined by Lindley (1956) is illustrated in protocol (i), but method (ii) used later is much to be preferred.

(i) *S*-Benzyloxycarbonylaminoethyl-L-cysteine (1·5 g) was dissolved in 1N sodium hydroxide (25 ml) at 0 °C, 1·7 ml benzyloxycarbonyl chloride added, and the reaction mixture shaken vigorously. After 10 min the solution was extracted once with ether to remove any excess benzyloxycarbonyl chloride, acidified with hydrochloric acid, and the product extracted into ether. Evaporation of the ether in a vacuum gave a colourless viscous oil. This is presumably the dibenzyloxycarbonyl derivative of *S*-aminoethyl cysteine, but was not further purified or characterized. The oil was heated with 1 ml thionyl chloride at 40 °C for 30 min. After evolution of $SO_2$ had ceased the excess thionyl chloride was removed by vacuum distillation (40 °C, $10^{-2}$ mm). On raising the temperature to 60 °C ($10^{-2}$ mm) the residue decomposed with liberation of benzyl chloride. On raising the temperature still further to 85–95 °C for 2 hr it further decomposed, liberating $CO_2$, and leaving a brown glass. This was dissolved in acetic acid with warming and after cooling was treated with 6N anhydrous hydrobromic acid in acetic acid for 2 hr. At the end of this time ether was added to complete precipitation of the product, which was separated by decantation and further washed with ether by decantation and finally filtered. It was then dissolved in water, extracted once with ether, and then treated with decolourizing charcoal, filtered, and neutralized to pH 4. The solution was concentrated to a small volume and precipitated by the addition of ethanol, the product recovered by centrifugation, and dried in a vacuum over sulphuric acid. Yield 0·3 g.

(ii) *S*-2-Benzyloxycarbonylaminoethyl-L-cysteine (3 g) was suspended in dry dioxan, heated to 40 °C, and a stream of phosgene passed in for 3 hr using a calcium chloride tube to prevent ingress of moisture. The dioxan was removed by evaporation in a vacuum at 50 °C, leaving behind a colourless viscous oil. This was heated at 105 °C and $10^{-2}$ mm for 3 hr to form a brown glassy residue of the polymer of *S*-2-benzyloxycarbonylaminoethyl cysteine, which was then treated as in (i) above. Yield 1·3 g. On hydrolysis with 6N HCl at 105 °C for 48 hr both samples gave only one ninhydrin positive spot on a one-dimensional paper chromatogram using phenol–ammonia. This had an $R_F$ of 0·82 agreeing with an authentic sample of *S*-aminoethyl cysteine and also gave a positive test with the iodoplatinate reagent (Consden, Gordon, and Martin 1946).

(*f*) *Carboxymethyl-2-aminoethyl Sulphide.*—Mercaptoacetic acid (4·6 g) was dissolved in water (50 ml) and bromoethylamine hydrobromide (10·5 g) added. 5N Sodium hydroxide (50 ml) was added and the solution let stand overnight. The solution was diluted with water to approx. 500 ml and passed through a column of " Zeo-Karb 225 " resin in the acid form, and washed well with water. The *product* was eluted with ammonia, the progress of the elution being followed by spot tests on filter paper using iodoplatinate solution. The eluate was concentrated in a vacuum to a thick gum which was finally induced to crystallize by leaving under dry ether. It was recrystallized by dissolving in twice its weight of water and adding 5 volumes of ethanol to the hot solution. Yield 5·5 g, m.p. 150 °C (Found: C, 35·7; H, 6·8; S, 23·8%. Calc. for $C_4H_9O_2NS$: C, 35·5; H, 6·7; S, 23·7%). The compound was tested for possible inhibition of trypsin by observing its effect on the rate of hydrolysis of toluene-*p*-sulphonyl-L-arginine methyl ester by trypsin, the reaction being followed in a pH-stat. With a substrate concentration of $0·7 \times 10^{-2}$M and a trypsin concentration of 0·0012 mg/ml at pH 8·0 and 30 °C, the rate of hydrolysis was not slowed down by the presence of $3·3 \times 10^{-2}$M carboxymethyl-2-aminoethyl sulphide.

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# SHORT COMMUNICATIONS

## A METHOD FOR INVESTIGATING POLYNUCLEAR COMPLEX FORMATION IN SOLUTION*

By D. D. PERRIN†

Stability constants for complex formation between any cation, M, and any ligand, L, can be determined by the theoretical treatment due to Bjerrum (1941). When some or all of the complexes, $M_xL_y$ (where $x$ and $y$ have the values : $m \geqslant x \geqslant 1$ ; $n \geqslant y \geqslant 1$ and $m$ and $n$ are the greatest number of metal ions and ligand molecules found in any complex), are present in the solution, Bjerrum's *formation function* becomes :

$$\bar{n} = \frac{\sum_{x=1}^{m} \sum_{y=1}^{n} y\beta_{xy}[M]^{x-1}[L]^{y}}{1 + \sum_{x=1}^{m} \sum_{y=1}^{n} x\beta_{xy}[M]^{x-1}[L]^{y}}, \quad \ldots\ldots\ldots (1)$$

where $\beta_{xy}$ is the overall stability constant of the complex $M_xL_y$. As the number of unknowns in the numerator and in the denominator depends on the product, $mn$, it is experimentally difficult to evaluate stability constants by this method when polynuclear complexes are present. Use of oxidation-reduction potentials (Perrin 1958) to calculate [M] makes some simplification possible, because it may readily be shown that

$$[M]_T/[M] = 1 + \sum_{x=1}^{m} \sum_{y=1}^{n} x\beta_{xy}[M]^{x-1}[L]^{y}, \quad \ldots\ldots\ldots (2)$$

where $[M]_T$ is the total concentration of M in the solution. However, this equation still contains $mn$ unknown constants.

In general, if polynuclear complexes are present, and especially where hydroxyl groups may also be bound to the metal ion, equations (1) and (2) become too complex for reliable constants to be obtained, and a simpler approach is necessary.

For solutions of varying total ferric iron concentration, at constant pH, ferrous ion and ligand concentration, the oxidation-reduction potentials vary according to the equation (Perrin 1959) :

$$\frac{2{\cdot}3026RT}{F.\partial E/\partial \log [Fe^{+++}]_T} = \frac{\sum\sum\sum_{x\ y\ z} x^2\beta_{xyz}[Fe^{+++}]^x[H^+]^{-y}[L]^z}{\sum\sum\sum_{x\ y\ z} x\beta_{xyz}[Fe^{+++}]^x[H^+]^{-y}[L]^z}, \quad \ldots\ldots (3)$$

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where $x,y,z$ refer to any complex, $Fe_x(OH)_yL_z$. Hence, the plot of $2.3026\,RT \log [Fe^{+++}]_T/F$ against $E$ will be a straight line of unit slope if only mononuclear complexes are present. When polynuclear complexes are also present, greater slopes should be found, the tangent to the curve at any selected value of $[Fe^{+++}]_T$ being the weighted mean value of $x$. It may readily be shown, by setting equation (**3**) equal to a constant and then differentiating with respect to $[Fe^{+++}]$, that if $x$ remains constant ($=c$, say) over a range of $[Fe^{+++}]_T$, only complexes containing $c$ ferric ions per molecule are present in significant amounts. If conditions are chosen such that this requirement is fulfilled over a range of pH and ligand concentrations, evaluation of the appropriate stability constants is considerably simplified, because one of the variables is eliminated. This treatment has recently been successfully applied to the ferric acetate system in which the complexes $Fe(CH_3COO)^{++}$ and $Fe_3(OH)_2(CH_3COO)_6^+$ are the main species present (Perrin 1959).

The procedure described above, and equation (**3**), should be applicable directly to any system in which metal ions are in equilibrium with electrodes of the same metal, including silver, cupric ion (with copper amalgam), and cadmium.

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## THE USE OF CARBODIIMIDES IN THE SYNTHESIS OF POLYPEPTIDES*

By J. H. Bradbury† and D. C. Shaw†

At the present time there is no method available for the preparation of high molecular weight non-random copolypeptides of the formula $NH_2$-(A-B)$_n$-COOH, where A and B are different amino acid residues. A number of different methods have been investigated, one of the most promising involving the formation of a peptide bond between an amino and carboxyl group in the presence of a carbodiimide (Sheehan and Hess 1955). The present paper is concerned with the polymerization of glycine and γ-benzyl-L-glutamate by various carbodiimides in an attempt to produce polypeptides of high molecular weight. Since the completion of this work Bruckner, Szekerke, and Kovács (1957) have reported the preparation of low molecular weight α-γ-polyglutamic acid by this method.

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Carbodiimides have been used in peptide synthesis for the formation of particular peptide bonds between protected amino acid or peptide derivatives (reviewed by Goodman and Kenner 1957). Khorana (1955) obtained yields of 25–50 per cent. of the desired, pure, product and separated an acyl urea (produced by a side-reaction) in amounts up to 18 per cent. Shankman and Schvo (1958) likewise isolated about 20 per cent. of an acyl urea, but they and other workers claim yields of 60–90 per cent. of the desired peptide derivative. Khorana (1955) proposed a mechanism for the reaction which includes the possible side reactions, the major one being rearrangement of the intermediate to give an acyl urea. He suggested that the latter reaction could be minimized by using solutions as concentrated as possible.

TABLE 1

PRODUCTS OF THE REACTION BETWEEN GLYCINE AND 1-ETHYL-3-(2-MORPHOLINYL-(4)-ETHYL)-CARBODIIMIDE IN AQUEOUS SOLUTION

| Temperature (°C) | Concentration of Glycine (%) | [Glycine]/[Carbodiimide] | Initial pH | Unreacted Glycine (%) | Soluble Reaction Products | | Concentrated Insoluble Polyglycine (%) |
|---|---|---|---|---|---|---|---|
| | | | | | Digly + Trigly (%) | Tetragly to Hexagly (%) | |
| 20–25 | 20 | 1 | 3 | 3 | 30 | 50 | 2 |
| 20–25 | 20 | 1 | 5 | 2 | 10 | 30 | 22* |
| 20–25 | 20 | 1 | 7 | 30 | 3 | 3 | 0 |
| 20–25 | 20 | 1 | 9 | 10 | 0 | 0 | 0 |
| 20–25 | 20 | 2 | 4 | 30 | 40 | 15 | 0 |
| 20–25 | 20 | 0·5 | 6 | 1 | 5 | 15 | 17* |
| 20–25 | 0·8 | 1 | 5 | 40 | 5 | 2 | 0 |
| 0 | 20 | 1 | 5 | 2 | 20 | 50 | 9 |
| 60 | 20 | 1 | 5 | 16 | 11 | 45 | 20 |

* Number average degree of polymerization equals $8 \pm 1$ as determined by titration of free carboxyl groups by the method of Meggy (1956).

In the production of polypeptides from carbodiimides and amino acids there is one other reaction, in addition to the side reactions discussed by Khorana (1955), which is capable of limiting chain growth. This is the possibility of cyclization of the growing peptide; a reaction which has been used by Wieland and Ohly (1957) to make a cyclic hexapeptide. Precipitation of the polymer from solution when a certain chain length is reached can also limit the size of the molecule in some cases.

The results for the polymerization of glycine are given in Table 1. Increasing the concentration of reagents from 0·8 to 20 per cent. greatly favours the production of polymer, presumably largely at the expense of acyl urea formation (see above). Variation of the ratio [glycine]/[carbodiimide] over a fourfold range shows that best results are obtained when this ratio is unity. The optimum pH for the reaction is about 5, and nothing is gained by performing the reaction

at 0 or 60 °C instead of room temperature. In the best case there was a 22 per cent. yield of insoluble polymer of DP about 8 as shown by end-group titration methods.

As shown in Table 2, polymerization of γ-benzyl-L-glutamate in water containing 10 per cent. dioxan yields about 20 per cent. of insoluble polymer. This material has a number average degree of polymerization ($DP_n$) of $5 \cdot 5 \pm 0 \cdot 2$ as obtained by titration of carboxyl end-groups and a weight average degree of polymerization ($DP_w$) of $9 \pm 3$, determined from measurement of the intrinsic viscosity. Since $DP_n < DP_w$ it is probable that the polymer is appreciably polydisperse and contains little, if any, cyclic material. The polymerization in dioxan-rich media using any of the three carbodiimides shown in Table 2 produces polymer of very low molecular weight (see below).

TABLE 2

PRODUCT OF THE REACTION BETWEEN γ-BENZYL-L-GLUTAMATE AND CARBODIIMIDES IN AQUEOUS DIOXAN (pH 4–6) AT 20–25 °C

| Compound | Concentration of γ-Benzyl-L-glutamate Hydrochloride (%) | [γ-Benzyl-L-Glutamate HCl]/[Carbodiimide] | Percentage Dioxan in Water | Yield of Insoluble Peptide (%) |
|---|---|---|---|---|
| 1-Ethyl-3-(2-morpholinyl-(4)-ethyl)carbodiimide | 6 | 1 | 10 | 20 |
| | 2·5 | 1 | 12 | 10 |
| | 2·5 | 1 | 75 | nil |
| | 3 | 1 | 83 | nil |
| 1-*cyclo*Hexyl-3-(2-morpholinyl-(4)-ethylcarbodiimide | 8 | 3·5 | 80 | nil |
| | 3 | 3·5 | 92 | nil |
| Di*cyclo*hexylcarbodiimide .. | 4 | 1 | 96 | nil |

Precipitation of polymer is a factor limiting the growth of polyglycine in aqueous solution and poly-γ-benzyl-L-glutamate in water containing 10 per cent. dioxan, but does not limit the growth of poly-γ-benzyl-L-glutamate in dioxan-rich media. Cyclization of peptides is probably not important in preventing chain growth in the polymerization of γ-benzyl-L-glutamate in water containing 10 per cent. dioxan. Therefore, the main factor limiting chain growth to 5–10 units in this work and about 15 in the preparation of polyglutamic acid (Bruckner, Szekerke, and Kovács 1957) must be other side reactions and in particular, acyl urea formation. It will not be possible to make high molecular weight polypeptide by the carbodiimide method until the nature of these side reactions is elucidated and some means devised to prevent their occurrence.

## *Experimental*

(*a*) *Preparation of Reagents.*—1-Ethyl-3-(2-morpholinyl-(4)-ethyl)carbodiimide and 1-*cyclo*hexyl-3-(2-morpholinyl-(4)-ethyl)carbodiimide were prepared by the method of Sheehan and Hlavka (1956, 1957). γ-Benzyl-L-glutamate was prepared by the first method of Hanby, Waley, and Watson (1950).

(*b*) *Polymerization of Glycine.*—Glycine (0·2 g) was dissolved in 1 ml of water containing a predetermined amount of HCl, 1-ethyl-3-(2-morpholinyl-(4)-ethyl)carbodiimide added at room temperature, and the initial pH measured with " Hydrion " indicator paper. The mixture was left overnight, insoluble polymer, if present, separated by centrifugation and weighed (see Table 1). The supernatant liquid was chromatographed for 3–4 days by the descending method on Whatman No. 1 paper using *n*-butanol–acetic acid–water (4 : 1 : 5) solvent. After spraying with ninhydrin and drying the paper discrete spots were obtained for the water-soluble peptides up to hexaglycine (in agreement with the results of Brockmann and Musso 1951) after which there was a tail of material stretching back to the origin. This tail was also observed when insoluble polymer was developed with the solvent. An approximate estimate of the percentage of glycine and each peptide present was made by the spot-dilution technique.

(*c*) *Polymerization of γ-Benzyl-L-glutamate.*—γ-Benzyl-L-glutamate hydrochloride (0·2 g) was dissolved in a dioxan-water mixture, the initial pH brought to 5 with HCl, the carbodiimide added, and the mixture left overnight. In the polymerizations made in water-rich media the polymer precipitated and was filtered off, dried, weighed, and its DP determined (see (*d*)). No precipitate of polymer was obtained from dioxan-rich media even when the solutions were poured into 95% ethanol, showing that any polymer produced must be of very low molecular weight (Blout and Karlson 1956). The solutions were chromatographed in one dimension on Whatman No. 4 paper, using *n*-butanol–acetic acid–water (4 : 1 : 5) solvent. In all cases there was a spot with $R_F = 0{\cdot}88$ which probably corresponds with the low molecular weight polymer ($R_F = 0{\cdot}92$) obtained by Mitchell, Woodward, and Doty (1957) but there was no evidence of higher molecular weight, helical polymer which remains at the origin according to these authors.

(*d*) *Degree of Polymerization of Poly-γ-benzyl-L-glutamate.*—The polymer was dissolved in chloroform and titrated with perchloric acid in methanol until the thymol blue indicator became red. It was then back titrated with sodium methoxide until the indicator became blue-grey. The titrations showed fewer amino than carboxyl end groups presumably due to end group cyclization of amino groups (Hanby, Waley, and Watson 1950; Mitchell, Woodward, and Doty 1957). Therefore $DP_n$ was calculated from the carboxyl end-groups and gave a value of $5{\cdot}5 \pm 0{\cdot}2$. The intrinsic viscosity of a sample in dichloroacetic acid was 0·067, which gave $DP_w = 9 \pm 3$, on application of Figure 1 of the paper by Mitchell, Woodward, and Doty (1957).

The authors wish to thank Dr. J. A. Maclaren for helpful discussions and the sample of di*cyclo*hexylcarbodiimide.

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# EFFECT OF pH ON THE DENATURATION OF LYSOZYME*

By A. N. Glazer†

Although the importance of pH as a major factor in determining the stability of proteins towards denaturing agents has been recognized for a long time, very few denaturation studies have been carried out over a wide pH range. Such studies offer information as to the details of the secondary and tertiary structure of proteins.

In the case of lysozyme, the effect of urea at pH 7·0 on the intrinsic viscosity, optical rotation, and enzymatic activity was examined by Leonis (1956), while the effect of guanidine hydrochloride at pH 4·8 on the viscosity and optical rotation was studied by Jirgensons (1952). In both cases, the authors reported changes in physical properties indicative of unfolding of the molecule.

In the present investigation, the effect of urea and guanidine hydrochloride on the viscosity and optical rotation of lysozyme has been studied over the pH range 2–10·5.

The measurements presented below were carried out at protein concentrations of 0·5 and 1 per cent. and the results have not been extrapolated back to infinite dilution. It may be noted here, however, that the slopes of the $\eta_{red.}$ *v.* concentration plots at pH 2·5, 6, and 10 for lysozyme over the concentration range 0·5–1 per cent. in 8M urea were found to be equal within experimental error.

In solutions of pH 4–8·5, the viscosity of lysozyme in 8M urea increased instantaneously from the value of 0·030 for the native protein to a value of about 0·046. Below pH 4, however, a further time-dependent viscosity increase took place, the rate of this change depended upon the pH—the lower the pH the faster the change.

In alkaline urea solutions, pH range 8·5–10·2, a similar situation obtained to that described above for the acid solutions. Here the slow changes could possibly be attributed partly to the slow hydrolytic splitting of some of the numerous cross-linking disulphide bonds.

The results shown in Figure 1 indicate that lysozyme is most stable in urea in the pH range 4–8.

The addition of cysteine (0·02M) was found to increase greatly the rate and extent of the viscosity changes in alkaline urea solutions of lysozyme (Table 1).

The results obtained with cysteine clearly show that disulphide bonds play a major role in maintaining lysozyme in its native configuration. This

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conclusion is not unexpected as the work of Fraenkel-Conrat *et al.* (1951) indicated that lysozyme consists of a single peptide chain cross-linked by disulphide bonds.

Since the range of maximum stability towards urea, pH 4–8, was rather unexpected for a protein of isoelectric point near pH 11, this effect was further investigated by studying the optical rotation changes of lysozyme in guanidinium

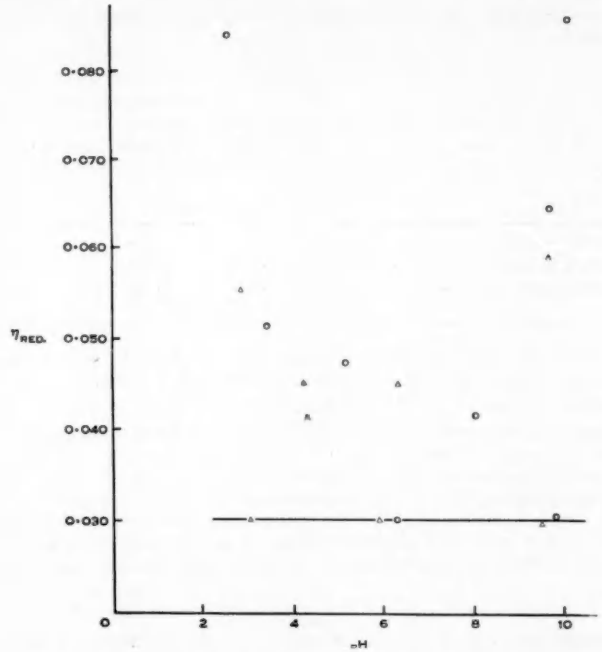


Fig. 1.—Viscosity of lysozyme (0·5 per cent.) in 8M urea, $\mu = 0{\cdot}1$, at the pH indicated, readings taken after 24 hr at 30 °C. The straight line represents viscosity of the native protein in the absence of urea, all other conditions as specified in text.

Δ Armour lysozyme.

○ Lysozyme prepared by the method of Alderton and Fevold (1946).

hydrochloride solutions at different pH values. (The laevorotation of lysozyme in 8M urea solutions did not vary significantly with pH over the range 2·7–9·6, the value being $-52° \pm 1$).

Results (Table 2) for 4M guanidine hydrochloride show that the optical rotation changes were found to be slowest and the final laevorotation values attained lowest in the pH region 4·0–8·0.

It would appear, therefore, that lysozyme exhibits its highest stability towards both urea and guanidine hydrochloride in the pH range 4–8.

## *Experimental*

(*a*) *Lysozyme.*—Lysozyme was prepared by direct crystallization from egg-white by the method of Alderton and Fevold (1946). This preparation was recrystallized three times. A sample of crystallized egg-white lysozyme from the Armour Laboratories (Lot no. 23547) was also used. The nitrogen content of the protein on a dry-weight, ash-free basis, was found to be $18{\cdot}5\pm0{\cdot}1$ g/100 g, and $E^{1\%}_{1\text{ cm}}$ at 280 mμ in 0·02N HCl was found to be 27·3 (published value: 27·2, Ehrenpreis and Warner (1956)).

(*b*) *Urea.*—Urea (C.P.) was recrystallized once from aqueous ethanol (70% v/v) at 50–60 °C and recovered by rapid cooling to —10 °C.

TABLE 1

EFFECT OF 0·02M CYSTEINE HYDROCHLORIDE ON THE VISCOSITY AND OPTICAL ROTATION OF 1 PER CENT. LYSOZYME IN UREA

All values taken after 24 hr at 30 °C; buffer: borate–KCl, $\mu=0{\cdot}12$

| Protein | pH | $\eta_{red.}$ | $[\alpha]_D^{30°}$ |
|---|---|---|---|
| Native protein .. .. .. | 9·6 | 0·032 | —50° |
| Protein in 8M urea .. .. | 9·6 | 0·065 | —52° |
| Protein in 8M urea + 0·02M cysteine | 9·5 | 0·145 | —108° |

(*c*) *Guanidine Hydrochloride.*—This compound was an Eastman Kodak reagent, twice recrystallized according to the method of Greenstein and Jenrette (1942).

(*d*) *Cysteine.*—L-Cysteine was obtained from Light and Co.

(*e*) *Preparation of the Reaction Mixture.*—The protein solution and the appropriate buffer-urea (or guanidine HCl) mixtures were brought to 30 °C. The solutions were then mixed and transferred by pipetting into the polarimeter tube or viscometer. The first reading on the polarimeter could be taken within 90 sec of starting the reaction and the first viscosity measurement within 3 min. The pH values of the reaction mixtures, measured by a Leeds and Northrup glass electrode at the beginning and end of each experiment, were constant to within 0·2 unit. The general procedure and standard buffers used were in accordance with the recommendation of Bates (1954).

TABLE 2

THE EFFECT OF pH ON THE OPTICAL ROTATION VALUES FOR LYSOZYME (0·5 PER CENT.) IN 4M GUANIDINE HYDROCHLORIDE

Values after 24 hr at 30 °C; buffers: HCl–NaCl, $\mu=0{\cdot}1$, for pH 0·7, 3·3, 4·3; $NaHPO_4$–$KH_2PO_4$, $\mu=0{\cdot}12$, for pH 6·6; borate–KCl, $\mu=0{\cdot}12$, for pH 8·5

| pH .. | 0·7 | 3·3 | 4·3 | 6·6 | 8·5 |
|---|---|---|---|---|---|
| $[\alpha]_D^{30°}$ .. | —89° | —81° | —73° | —60° | —70° |

(*f*) *Viscosity.*—The Ostwald type viscometers were maintained at $30\pm0{\cdot}03$ °C. The working volumes were about 12 ml and the flow times for water approximately 45 sec. The dimensions of the capillary were 0·8 mm (i.d. approx.) by 30 cm (length). The results are expressed in terms of the reduced viscosity which is defined as

$$\eta_{red.}=\frac{1}{C}[\eta_{rel.}-1],$$

where $C$ is the protein concentration in g/100 ml of solution and $\eta_{rel.}$ is relative viscosity. Optical rotation was measured at $30\pm0{\cdot}01$ °C in a Schmidt and Haensch polarimeter, using a 1 dm polarimeter tube.

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## CORRIGENDA

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Page 32, line 7 from the bottom: *For* (0·2 mm diameter) *read* (0·02 mm diameter).
Page 37, line 7 from the top: *For* square *read* brace.
Page 49, last line: *For* (IV) $\xrightarrow{+H^+}$ (V) *read* (IV) $\xrightarrow{-H^+}$ (V).
Page 51, line 12 (reaction (8)): Add above the first carbon atom V.
Page 53, line 8 from bottom: *For* VIII *read* XIII.
Page 98: *For* the first structure (III) *read* (II).

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